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# Production of Universal Red Blood Cells via Enzymatic Conversion

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# Production of Universal Red Blood Cells via Enzymatic Conversion

## **Abstract**

Red blood cell (RBC) transfusion units are considered one of the most essential healthcare components in the world. In the United States alone, approximately 21 million transfusion units are required every year. Despite this high demand, RBC units are becoming increasingly scarce since only a fraction of eligible donors provide RBCs for medical use. Additionally, RBC transfusions are limited to immune compatibility in patients, making it difficult to serve all patients with such a limited supply. This proposed design provides a method in which RBCs of any blood group can be converted into the universal blood type, O, to eliminate any concerns regarding blood type compatibility between donor and patient. This conversion process uses bacterial glycosidases to cleave the sugar groups on the surface of RBCs that defines our blood type. This process will help increase hospitals' supply of readily usable RBCs for any situation while also providing a solution to hospitals' struggle to use their blood bags before they expire.

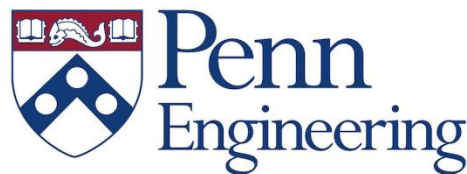
This proposal seeks to design a start-up scale plant that will both prepare the glycosidases needed for the treatment process and execute the conversion. This project design expects a production capacity of 200,000 transfusion units of successfully converted RBCs per year and will be located in Medford, MA. With an initial investment of \$25.6 million, the design yields a twelve-year net present value of \$8,461,700 and has an investor's rate of return of 21.73%. A limited twelve-year lifespan was chosen in an attempt to more accurately represent the lifespan of a start-up and to more strictly analyze its financial feasibility. The proposed project is forecasted to breakeven in early 2028, at the beginning of its eighth year of its operation, with a return on investment of 17.17%. With initial evidence of profitability, this project design is recommended. Furthermore, the financial analysis performed in this report limits the scope of this project to satisfying the blood demands of one major hospital in a metropolitan area. In reality, however, it is expected that the start-up will expand to other major hospitals or blood collecting organizations within the first several years of operation, further increasing its potential value. It should be noted, however, that investors exercise caution as the blood market has been in constant flux for the past seven years, making it difficult to predict how valuable RBC transfusion units will be compared to other blood components. The process should be executed only if an acceptable pricing can be established to sustain the large costs associated with guaranteeing endotoxin and contamination free products.

## **Disciplines**

Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering



April 18, 2017  
University of Pennsylvania  
School of Engineering and Applied Science  
Department of Chemical and Biomolecular Engineering  
220 S. 33rd Street  
Philadelphia, PA 19104



Dear Professors Vrana and Wattenbarger:

Enclosed is a process design to produce universal type O red blood cells (RBCs) via enzymatic conversion on a startup scale. Type O RBCs, as the type of red blood cells most used in blood transfusions, are in increasingly high demand and increasingly short supply. Successful FDA approval is assumed for eventual market launch; it is hoped that our product will advance movement towards a universal blood supply.

The design of the universal enzymatically converted (ECO) RBC involves two processes: growth of the ECO-enzymes in *E. coli* and subsequent purification, and enzymatic conversion treatment of the RBCs themselves. Upstream operations in the first process include an inoculum bioreactor followed by a growth bioreactor and two production fermenters wherein *E. coli* grows in high density in high-salt media supplemented with glucose, kanamycin, and chloramphenicol. Downstream operations for the first process include disk stage centrifugation, high-pressure homogenization to release cytoplasmic ECO-zymes, cobalt affinity, HIC, and endotoxin affinity chromatography to ensure nearly complete removal of cell debris and endotoxins. Ultrafiltration/diafiltration and bulk microfiltration follow to resuspend and sterilize the isolated ECO-zymes. The RBC conversion process involves centrifugation of unconverted RBCs, incubation of RBCs and ECO-zymes in a WAVE bioreactor to effect conversion, centrifugation, diafiltration, and cryogel affinity chromatography to separate enzyme from converted ECO RBCs.

The process in theory yields 22.91 kg ECO-A-zyme, 0.376 kg ECO-B-zyme, and 200,000 units of ECO RBCs produced annually. Internal rate of return (IRR) is 21.73%, net present value (NPV) is \$8.46 million in 2019, and return on investment (ROI) is 17.17%. Our process requires \$27.3 million working capital for a 12-year facility life span.

All calculations within stem from suggestions from Dr. Jeffrey Cohen, Dr. Robert Luo, Dr. Daniel Green, Mr. Gary Sawyer, and other industrial consultants or from the literature. Please do not hesitate to reach out should any questions or the need for supplemental information arise.

Sincerely,

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Carly Catella

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Rachel Coler

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Brandon Hayes



# **Production of Universal Red Blood Cells via Enzymatic Conversion**

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**April 17<sup>th</sup>, 2018**



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# **Section 1:**

# **Abstract**

## 1. Abstract

---

Red blood cell (RBC) transfusion units are considered one of the most essential healthcare components in the world. In the United States alone, approximately 21 million transfusion units are required every year. Despite this high demand, RBC units are becoming increasingly scarce since only a fraction of eligible donors provide RBCs to for medical use. Additionally, RBC transfusions are limited to immune compatibility in patients, making it difficult to serve all patients with such a limited supply. This proposed design provides a method in which RBCs of any blood group can be converted into the universal blood type, O, to eliminate any concerns regarding blood type compatibility between donor and patient. This conversion process uses bacterial glycosidases to cleave the sugar groups on the surface of RBCs that defines our blood type. This process will help increase hospitals' supply of readily usable RBCs for any situation while also providing a solution to hospitals' struggle to use their blood bags before they expire.

This proposal seeks to design a start-up scale plant that will both prepare the glycosidases needed for the treatment process and execute the conversion. This project design expects a production capacity of 200,000 transfusion units of successfully converted RBCs per year and will be located in Medford, MA. With an initial investment of \$25.6 million, the design yields a twelve-year net present value of \$8,461,700 and has an investor's rate of return of 21.73%. A limited twelve-year lifespan was chosen in an attempt to more accurately represent the lifespan of a start-up and to more strictly analyze its financial feasibility. The proposed project is forecasted to breakeven in early 2028, at the beginning of its eighth year of its operation, with a return on investment of 17.17%. With initial evidence of profitability, this project design is recommended. Furthermore, the financial analysis performed in this report limits the scope of this project to satisfying the blood demands of one major hospital in a metropolitan area. In reality, however, it is expected that the start-up will expand to other major hospitals or blood collecting organizations within the first several years of operation, further increasing its potential value. It should be noted, however, that investors exercise caution as the blood market has been in constant flux for the past seven years, making it difficult to predict how valuable RBC transfusion units will be compared to other blood components. The process should be executed only if an acceptable pricing can be established to sustain the large costs associated with guaranteeing endotoxin and contamination free products.

# **Section 2: Introduction**

## 2. Introduction

### 2.1. Project Background

Blood is an essential part of healthcare, listed on the World Health Organization's List of Essential Medicines<sup>1</sup>. Its components are always in high demand, of which red blood cells (RBCs) are the most used in transfusions. Globally, around 85 million units of packed RBCs are used annually, of which approximately 21 million are used in the United States alone<sup>2,3</sup>. Despite the importance of RBC transfusions, the compatibility of donated blood, based on the ABO group system, with patients' immune systems often hinders treatment efficiency<sup>4</sup>. Plasma of blood group A individuals contains naturally occurring antibodies to the B antigen present on group B RBCs, marking them for destruction. Similarly, plasma of blood group B contains antibodies against A antigen. This means that individuals with blood of group A cannot receive transfusions from group B blood and vice-versa. Otherwise, patients will become anemic and suffer immune responses<sup>4</sup>.

Type-O RBCs, however, do not contain A or B antigens and can be safely transfused into patients of any blood type, as shown in Figure 2.1. For this reason, type-O blood is considered the “universal” blood type and is in high demand by blood banks and hospitals<sup>4</sup>.

	Donor's Blood Type							
	O-	O+	B-	B+	A-	A+	AB-	AB+
AB+	✓	✓	✓	✓	✓	✓	✓	✓
AB-	✓		✓		✓		✓	
A+	✓	✓			✓	✓		
A-	✓				✓			
B+	✓	✓	✓	✓				
B-	✓		✓					
O+	✓	✓						
O-	✓							

**Figure 2.1.** Blood type compatibility chart. Note that type-O donors are able to donate to any other RBC type when not considering the Rh factor.

<sup>1</sup> World Health Organization. WHO Model List of Essential Medicines, 19<sup>th</sup> List [Internet]. 2015 [cited 2018 Apr 15]. Available from: <http://www.who.int/medicines/publications/essentialmedicines/en/>

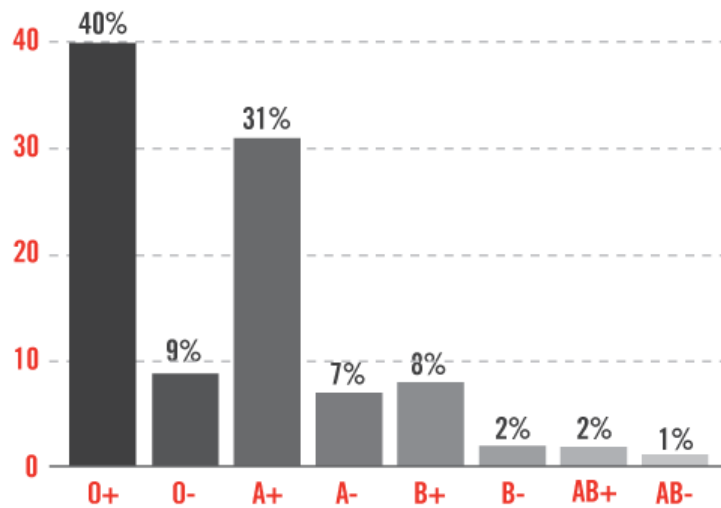
<sup>2</sup> American Red Cross. Blood Facts and Statistics [Internet]. 2018 [cited 2018 Apr 15]. Available from: <https://www.redcrossblood.org/learn-about-blood/blood-facts-and-statistics>

<sup>3</sup> Carson JL et al. Red blood cell transfusion: a clinical practice guideline from the AABB. *Annals of Internal Medicine* 2007; 157: 49–58.

<sup>4</sup> Liu QP et al. Bacterial glycosidases for the production of universal red blood cells. *Nature Biotechnology* 2007; 25(4): 454–464.



Donations from type-O donors are highly sought after, but a significant amount of type-A, B and AB are also collected to be used with compatible recipients. However, a disproportionate amount of type-O blood is used in trauma or situations with significant incompatibility risks, which leads to a surplus of type-A, B and AB transfusion units<sup>5</sup>. RBCs are only stable and efficacious for 42 days when refrigerated at 4°C, limiting their long-term viability and monetary value<sup>6</sup>. Improper blood management strategies leads to a significant number of unused packed RBC units, which tends to range from 20 to 30% of a hospital's entire blood supply<sup>7</sup>.



**Figure 2.2.** Blood type distribution in the United States.

Although 49% of the population of the United States has type-O RBCs, as shown in Figure 2.2, only an estimated ten percent of the eligible U.S. population (38% of the total population) donates blood<sup>2</sup>. In addition, the already limited number of willing donors has been affected by changes in blood transfusion policies in the country in recent years. After a 15-year steady increase in blood transfusions, the number of transfusions performed has been decreasing since 2011 due to more stringent requirements for patients to receive transfusions<sup>8</sup>. Hospitals have begun limiting transfusions to patients only when their hemoglobin levels drop below 10 grams per deciliter, nearly a two-fold change compared to the time prior to this policy's implementation. This was done to minimize hospital stays; to prevent the risk of infection, allergic reaction, or

<sup>5</sup> Strobel E. Hemolytic transfusion reactions. *Transfus. Med. Hemother.* 2008; 35(5): 346-353.

<sup>6</sup> Miraflor E et al. Emergency uncrossmatched transfusion effect on blood type alloantibodies. *J. Trauma Acute Care Surg.* 2012; 72(1): 48-52

<sup>7</sup> Kurup R et al. A study on blood product usage and wastage at the public hospital, Guyana. *BMC Res. Notes* 2016 Jun; 9: 307.

<sup>8</sup> Mohebbi Far R et al. Determination of rate and causes of wastage of blood and blood products in Iranian hospitals. *Turk. J. Haematol.* 2014 Jun; 31(2): 161-167

contamination; and lessen treatment and operating costs imposed on hospitals for performing unnecessary transfusions. As a result of this policy change, hospitals began to use significantly fewer RBC transfusion units, leaving them with a surplus of transfusion units that only have a shelf life of 42 days<sup>8</sup>. This eventually affected the blood collection industry, as sales began to drop since hospitals now required fewer transfusion units. It also began to affect public awareness on the issue, and both blood banks and the Red Cross began to receive fewer active donors.

The policy change has constrained current blood supplies significantly without properly considering new health and medical treatment challenges in the present and upcoming years. According to the World Health Organization, blood-intensive procedures are expected to grow, such as solid organ transplants, hematopoietic stem cell transplants, and aggressive chemotherapy against cancer, all of which will require increased blood supplies. The prevalence of cancer in the American population, which is expected to surpass heart disease as the main cause of death in the United States by as early as 2030, is specifically considered an area where RBC transfusion units are predicted to be needed in a greater supply than what is currently available. According to the American Cancer Society, more than 1.69 million people were diagnosed with cancer in 2017. Many of them will need blood, sometimes daily, during their chemotherapy treatment. Lastly, the continually growing population over 65 years of age is also expected to increase demand for blood to combat anemia, aggressive immunogenic responses, and other health complications<sup>9,10</sup>.

Surgery cancellation at hospitals has become increasingly more common across the United States and is often reported in mainstream media. Blood supply scarcity announcements from hospitals are also becoming more frequent. An example of the poor blood supply occurred in Puerto Rico in February 2016. Due to the growing prevalence of the Zika virus and FDA concerns of it being possibly transmitted via transfusion, blood collections in Puerto Rico ceased until nucleic acid testing could be implemented under investigational new drug protocols<sup>11</sup>. This forced the territory to eventually import blood and packed RBCs.

The global blood collection market size was valued at \$7.6 billion in 2015, to which RBC transfusions contributed \$1.8 billion<sup>12</sup>. Economic analyses predict that this market is projected to

<sup>9</sup> Schulson M. Blood transfusions decline 2011-14, study finds [Internet]. Cornell Chronicle 2018. Web. <http://news.cornell.edu/stories/2018/02/blood-transfusions-decline-2011-14-new-study-finds>

<sup>10</sup> Olson ML, Clausen L. Modifying the red cell surface: towards an ABO-universal blood supply. *British Journal of Haematology* 2007;140,3-12.

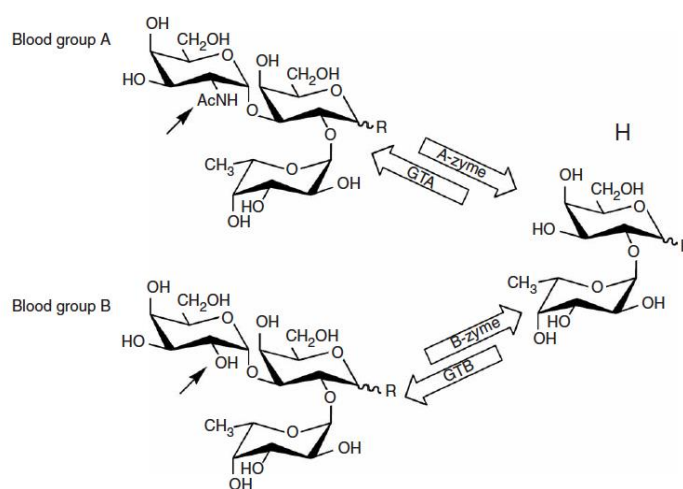
<sup>11</sup> Sullivan L. Blood Industry Gets A Transfusion From An Aging Population. BCC Research 2017 [cited 2018 Apr 15]. Available from: <http://blog.bccresearch.com/blood-industry-gets-a-transfusion-from-an-aging-population>

<sup>12</sup> Ellingson KD et al. Continued decline in blood collection and transfusion in the United States-2015. *Transfusion* 2017 Jun; 57(2): 1588-1598

grow by 6.1% by the 2021-2022 period due to the increasing prevalence of infectious disease and non-communicable disease, cancer, accidents, and trauma cases. This validates that there is a need for improve the currently available supply. This proposal seeks to develop a platform in which universal, type-O RBCs can be prepared from any other blood cell type, taking advantage of the surplus of transfusion units that large hospitals usually have. These enzymatically converted type-O (ECO) RBCs will be usable in all patients, will function exactly the same as type-O cells, and not generate adverse reactions in patients.

## 2.2. Basis for Project and its Biochemistry

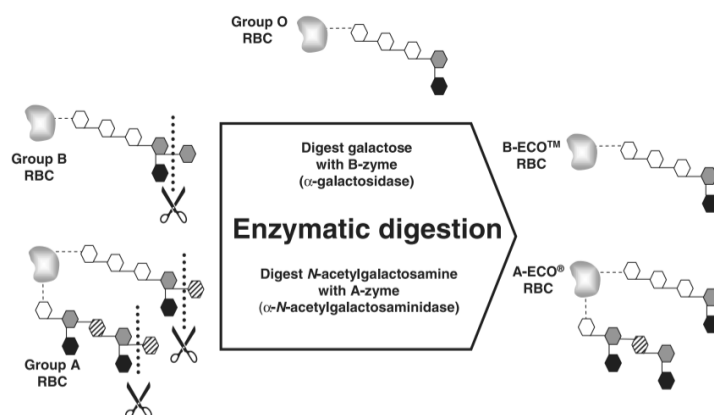
The ABO blood group system, which determines all transfusion compatibility, categorizes blood based on the presence or absence of the A and B blood group antigens on the surfaces of RBCs. The carbohydrate structures that make RBCs type-A, B, or O are designated A, B, and H and are present at oligosaccharide chain termini of glycolipids and glycoproteins on RBC surfaces<sup>4</sup>. A antigen structure and specificity are determined by a terminal  $\alpha$ -1,3-linked N-acetylgalactosamine (GalNAc); B antigen specificity is derived from an  $\alpha$ -1,3-linked galactose (Gal). O RBCs are capped with the H antigen containing a terminal  $\alpha$ -1,2-linked fucose (Fuc)<sup>4</sup>. Enzymatic removal of the immunodominant monosaccharides from type-A and B RBCs respectively, will uncover the H antigen and yield universal type-O RBCs. This biochemical reaction is shown in Figure 2.3.



**Figure 2.3.** Biochemical reaction showing the terminal sugar groups that create human blood group A and B antigens on RBCs. Note that R in the above image is shorthand for RBC.

In the 1980s, a procedure was established to convert group B RBCs into group O by using an  $\alpha$ -galactosidase derived from green-roasted coffee beans (*Coffea canephora*). This coffee bean-derived enzyme completed phase I and phase II clinical trials, and the treated RBCs were shown to be safe and functional. Unfortunately, the amount of enzyme needed to treat a single unit of group B RBCs was too high to create a cost-effective process (1 to 2 grams/unit packed RBCs) with which these universal blood cells could be manufactured. Additionally, the coffee bean-derived  $\alpha$ -galactosidase could only treat group B RBCs, not group A, limiting its usability<sup>4</sup>.

In 2007, however, another research group identified two prokaryotic glycosidase gene families that could be used to potentially convert any RBC type into the universal type: one containing  $\alpha$ -N-acetylgalactosaminidase (GH109), which has high activity with group A antigens, and another containing  $\alpha$ -galactosidase (GH110), which targets group B antigens<sup>4</sup>. Additional progress in the field was made in 2015 when the family 98 glycoside hydrolase from *Streptococcus pneumoniae* SP3-BS71 was found to cleave the entire terminal trisaccharide antigenic determinants of both A and B antigens on the RBC surface<sup>13</sup>.



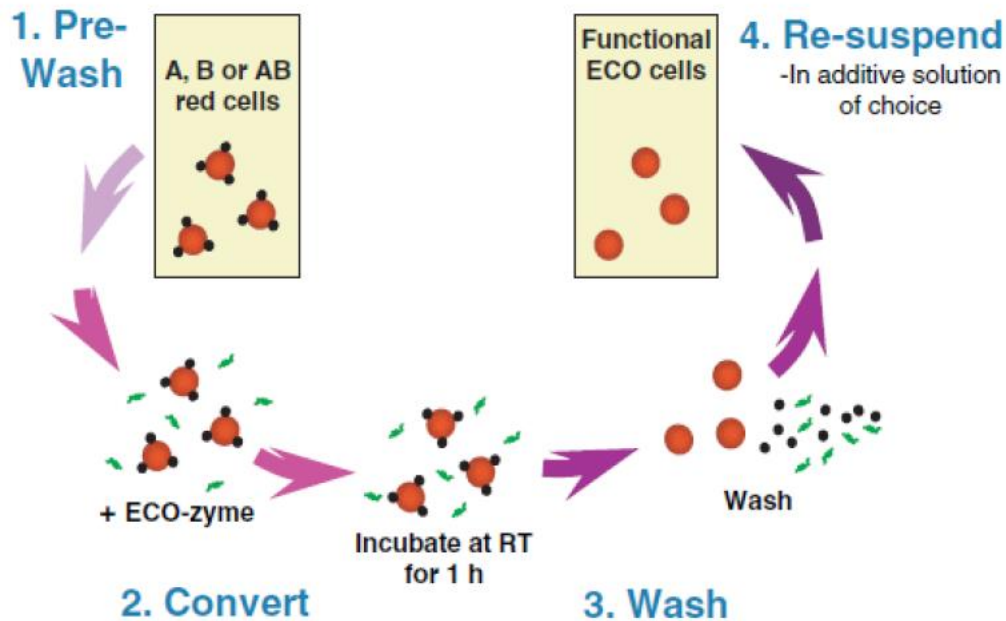
**Figure 2.4.** Illustration of the specific cleavages of A and B antigens and the uncovering of H antigen via ECO-A and ECO-B enzymes, respectively. Color code for monosaccharides (hexagon symbols): fucose (black), galactose (grey), N-acetylgalactosamine (hatched) and precursor chain constituents (white).

The  $\alpha$ -N-acetylgalactosaminidase and  $\alpha$ -galactosidase genes will be used to enzymatically convert groups A and B RBCs into ECO RBCs. These genes will be inserted into *E. coli* to produce

<sup>13</sup> Kwan DH et al. Toward efficient enzymes for the generation of universal blood through structure-guided directed evolution. *J. Am. Chem. Soc.* 2015; 137(7): 5965-5705

ECO-A and ECO-B enzymes for downstream treatment of RBCs. The cleavage process is detailed in Figure 2.4, which highlights the targets that the ECO-zymes use to cleave the A and B antigens to form the blunt H sugar group found on type-O RBCs. The resulting ECO RBCs will have the terminal H antigen that is universal to all patients.

The conversion process that transforms group A and B RBCs to ECO RBCs is quick and efficient. The incubation step is an hour at room temperature, and the buffers for both ECO-zymes are the same. Approximately 60 mg of A ECO-zyme is needed to treat a liter of group A RBCs, and 2 mg of B ECO-zyme is needed to treat a liter of group B RBCs.



**Figure 2.4.** General schematic of process for converting type-A, B, or AB red blood cells to universal RBCs.

### 2.3 Introduction to Project Process Synthesis

The enzymatic treatment process of RBCs can be separated into upstream production of the ECO-zymes, and the downstream conversion of the RBCs. In the upstream phase, the ECO-zymes are produced through *E. coli* growth in a three-stage bioreactor system. The cells are lysed using a microfluidizer to release the ECO-zymes, the broth is centrifuged, and the debris is discarded. The ECO-zymes are then separated and purified through three chromatography stages.

The first stage is an affinity chromatography column, which uses His<sub>6</sub>-tag technology to bind to and separate the ECO-zymes from the other extracellular proteins. The last two columns, a hydrophobic interaction chromatography column and an affinity column, are used to remove endotoxins. The ECO-zyme product will be tested for endotoxins with the Lonza Endotoxin kit.

The downstream treatment of the RBCs uses the ECO-zymes produced upstream to enzymatically convert type-A and B RBCs into non-immunogenic type-O RBCs. After the RBC units are emptied and the packaging buffer is removed the RBCs are sent to a wave bioreactor for conversion. The conversion process takes one hour at room temperature, with ECO-zymes and a neutral buffer. Centrifugation and filtration are used to separate and remove the ECO-zymes from the RBCs. Cryogel based affinity chromatography is used to capture and remove unconverted RBCs, which could cause an immune response if left in the product. Finally, the RBCs are resuspended in packaging buffer and filled into blood bags for distribution. Quality control measures are in place to test and confirm the conversion of the RBC units prior to distribution.

## 2.4. Project Charter

<b>Project Name</b>	Production of Universal Red Blood Cells via Enzymatic Conversion
<b>Leaders</b>	Carly Catella, Rachel Coler, Brandon Hayes
<b>Project Scope</b>	<u>In Scope:</u> <ul style="list-style-type: none"> <li>• Manufacturing process for annual production of 205 g of <math>\alpha</math>-N-acetylgalactosaminidase and 189 g of <math>\alpha</math>-galactosidase, beginning with inoculum preparation and concluding with large-scale fermentation</li> <li>• Post-manufacturing purification and separation of product enzymes using multiple chromatography and filtration steps</li> <li>• Testing to verify enzyme product, determine purity, and quantify endotoxin concentration to guarantee patient safety when using treated RBCs</li> <li>• Production of 200,000 RBC transfusion units/year with selected enzymes</li> <li>• Establishment of sterile unpacking/packaging mechanism of RBCs to be processed to minimize human error and risk of contamination</li> <li>• Adherence to current health and safety regulations for blood products</li> <li>• Observance of process integrity and compliance by adhering to current good manufacturing practices (cGMP)</li> </ul>
	<u>Out of Scope:</u> <ul style="list-style-type: none"> <li>• Removal of Rh factor to account for positive/negative typing of RBCs</li> <li>• Research and development (performed in laboratory): <ul style="list-style-type: none"> <li>- General characterization and kinetics of <math>\alpha</math>-galactosidase and <math>\alpha</math>-N-acetylgalactosaminidase</li> <li>- Specific design of hyperbaric chamber for blood bag emptying</li> <li>- General characterization of cryogel polymer matrix used in cryogel-based affinity chromatography</li> </ul> </li> <li>• Long-term storage of RBCs at customers' facilities</li> <li>• FDA approval for a Biological License Application (BLA) for conversion process and blood product</li> <li>• Establishment, funding, and success of clinical trials</li> <li>• Pilot plant study to analyze cell growth rates and optimal conditions</li> </ul>
<b>Deliverables</b>	<u>Business Opportunity Assessment</u> <ul style="list-style-type: none"> <li>• What are the current and projected markets for type-O RBCs?</li> <li>• What added value does our process provide over traditional RBC sources?</li> </ul> <u>Technical Feasibility Assessment</u> <ul style="list-style-type: none"> <li>• Is it feasible to produce enough enzyme for 200,000 ECO RBC units/year?</li> <li>• Is it feasible to convert 200,000 units of RBCs/year with minimal RBC lysis and build a sustainable business model?</li> </ul> <u>Manufacturing Capability Assessment</u> <ul style="list-style-type: none"> <li>• Will the process require significant capital investment to produce enzymatically converted RBCs?</li> <li>• Will the process meet FDA requirements for blood products?</li> </ul>
<b>Timeline</b>	Facility, process design, economic analysis will be completed in four months.

# **Section 3:**

# **Innovation Map**



### 3. Innovation Map

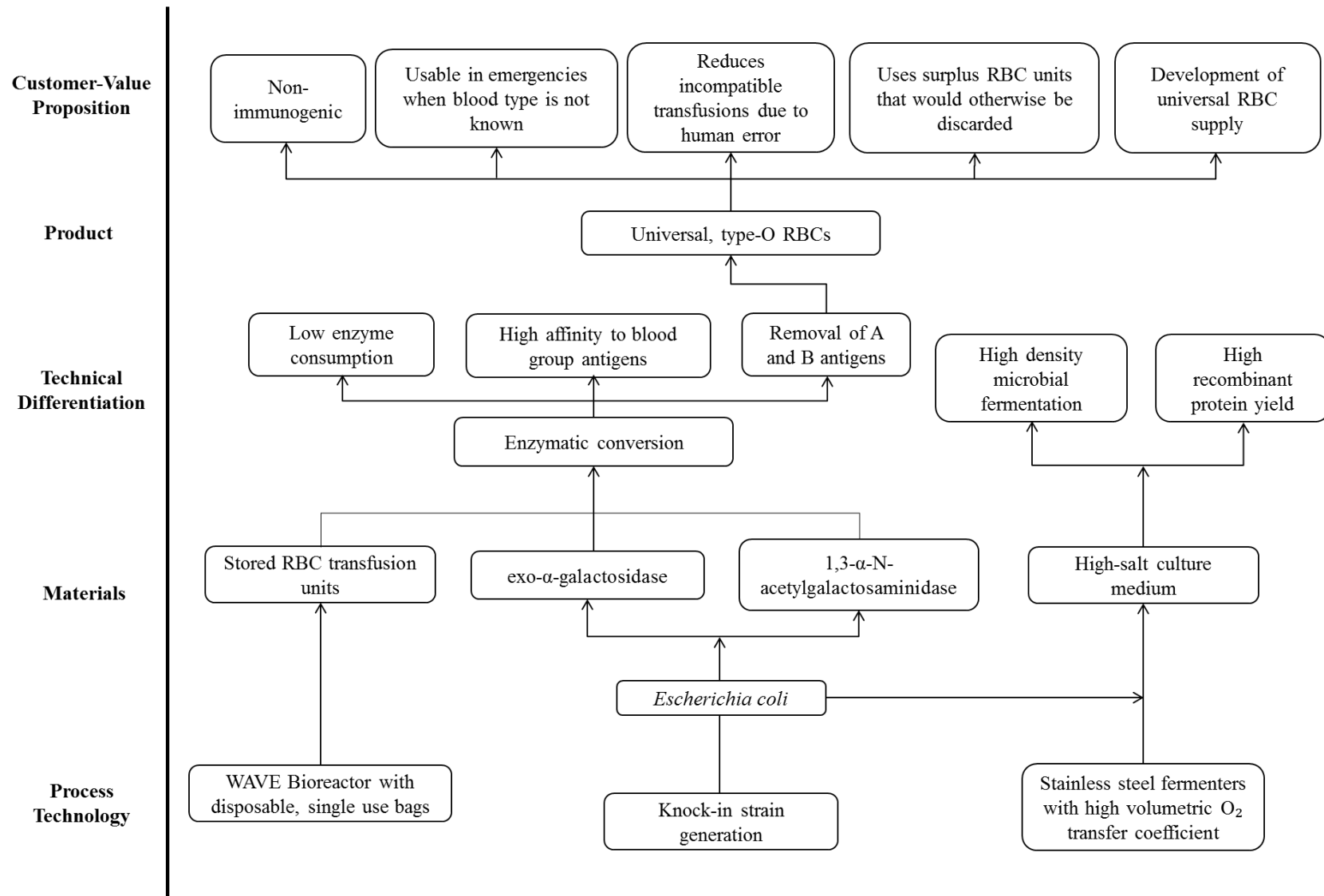
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Figure 3.1 shows the innovation map for enzymatically converted RBC units. Customers value the non-immunogenic nature of type-O RBCs because they can be used in situations where the blood type of the patient is not known<sup>4</sup>. Traditional RBC units come in a variety of blood types, and only limited supplies of the non-immunogenic type-O are available<sup>10</sup>. Potential customers are currently limited to the fluctuating supplies of type-A, B, AB, and O red blood cells available, which have become more limited in the past decade due to transfusion medicine policy changes<sup>9</sup>.

Enzymatically converted universal RBCs utilize the traditional RBC supply chain but add value by removing the immunogenic antigens from the surface of the RBCs with bacterial glycosidases. The antigen-cleaving glycosidases,  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase, function with high affinity and low consumption, making them suitable choices for large-scale RBC treatment processes<sup>1</sup>. Enzymatically converted universal RBCs are poised to enter the market to fill the need for a greater and steadier supply of non-immunogenic units. In regards to potential competition, recent academic researchers presenting at stem cell research conferences have claimed that they engineered an immortalized hematopoietic stem cell line that can continuously produce red blood cells<sup>14</sup>. However, the production rates of this stem cell line are still unknown, making it uncertain that they can produce RBCs at a fast enough rate to satisfy global demand. Additionally, these RBCs have not yet undergone pre-clinical or clinical trials yet, indicating that their potential use in human patients is still several decades away. The RBC treatment process described here has already undergone several trials, in which certain of its weaknesses were addressed and have since been occurred<sup>1,2</sup>. Prior data on the efficacy, performance, and immunogenicity of ECO RBCs should allow the process and product to move through Phase I-III clinical trials and the FDA approval process more smoothly. Thus, it can be concluded that there are currently no other RBC products on the market that can offer non-immunogenic properties in large quantities, so there would be no competition in this market segment.

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<sup>14</sup> Shah S, Huang X, and Cheng L. Concise review: stem cell-based approaches to red blood cell production for transfusion. *Stem Cells Transl. Med.* 2014 Mar; 3(3): 346-355.



**Figure 3.1.** Innovation map for ECO RBC transfusion units. Enzymatically converted RBCs will be prepared as ready-to-administer so they can directly enter the market due to their ability to meet market needs and differentiation from traditional RBC units.

# **Section 4:**

# **Concept Stage**

## 4. Concept Stage

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### 4.1. Market Analysis

The global blood collection market size was valued at \$7.6 billion in 2015, to which RBC transfusions contribute \$1.8 billion<sup>15</sup>. This market is projected to grow at 6.1% by the 2021-2022 period. RBC demand is also expected to increase due to the increasing prevalence of infectious disease and non-communicable disease, cancer, accidents, and trauma cases<sup>10</sup>. The number of blood-intensive procedures performed, such as solid organ transplants, hematopoietic stem cell transplants, and aggressive chemotherapy against cancer, is also expected to grow; all of these treatments require increased blood supplies<sup>10</sup>. Lastly, the continually growing population over 65 years of age is also expected to increase demand for blood<sup>10</sup>.

Despite the need for packed RBCs in transfusion medicine, supply has decreased significantly, especially in the last decade. Although an estimated 38% of the US population is eligible to donate blood at any given moment, less than 10% of that eligible population donates<sup>2</sup>. Another problem for transfusion medicine is how quickly packed RBCs expire. Typically, any given unit of RBCs is only usable for 42 days. Due to their short life span, packed RBC units are constantly being discarded, forcing hospitals to replenish their stock. It has become increasingly difficult to maintain inventories, because demand is expected to grow while supply is being hindered by stricter donation regulations and fewer donors.

The current industry standard for RBC unit production is whole blood donation, separation, purification, and repackaging. Supply is dependent on the donors' blood types, willingness to donate, and limited shelf life of the packaged RBC unit. Certain hospital wards, such as trauma wings and emergency rooms, will transfuse O negative RBCs until properly cross matched blood is available when there is no time to type a patient's blood<sup>16</sup>. This leads to a disproportionate use of O RBC units, which contributes to the wastage of the lesser used blood types, A, B, and AB. Approximately 78% of wasted RBC units are thrown out due to time expiry<sup>17</sup>.

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<sup>15</sup> Ellingson KD et al. Continued decline in blood collection and transfusion in the United States-2015. *Transfusion* 2017 Jun; 57(2): 1588-1598.

<sup>16</sup> Miraflor E et al. Emergency uncrossmatched transfusion effect on blood type alloantibodies. *J. Trauma Acute Care Surg.* 2012; 72(1): 48-52.

<sup>17</sup> Miraflor E et al. Emergency uncrossmatched transfusion effect on blood type alloantibodies. *J. Trauma Acute Care Surg.* 2012; 72(1): 48-52.

Strobel E. Hemolytic transfusion reactions. *Transfus. Med. Hemother.* 2008; 35(5): 346-353.

Additional concerns for RBC unit transfusions are the liabilities associated with transfusion incompatibilities, which can lead to adverse events, such as acute hemolysis. Reactions can range from mild to fatal, with rates of fatal reactions estimated to be between 1:70000 and 1:100000 units transfused<sup>5</sup>. Each transfusion incompatibility event can cost hospitals and their respective insurance companies upwards of \$500,000<sup>18</sup>. The most common cause of transfusion incompatibility events is mismatched blood types from the donor and patient, which can be caused by systemic problems and/or human error<sup>5</sup>. The former may be addressed by implementing more rigorous quality assurance practices, while the latter is harder to control for, and will likely continue to be a problem in the current system.

A universal RBC supply could solve both the wastage and liability concerns associated with the standard RBC transfusion practices. Currently, there is no universal option for blood products. Work has been done to produce RBCs *ex vivo* using differentiated stem cells. However, these RBCs lack adult hemoglobin, their production relies on animal serum, and scale up has not been successful thus far<sup>14</sup>. Enzymatically converted universal RBCs would be the first universal blood product to go to market in this sector and would be highly desirable in situations where blood typing is performed on a limited scale or there are incompatibility risks.

## 4.2. Customer Requirements

Hospital systems are the primary users of RBC units and typically source them from regional suppliers, the Red Cross, and internal blood drives.<sup>19</sup> The enzymatically converted RBC units will directly replace RBC units sourced from these three suppliers while providing additional benefits, such as reduced liability and higher usage efficiency. In addition, these units must meet the same safety guidelines set by the Food and Drug Administration (FDA) that traditional RBC units meet.

In general, RBC units must be processed in a way that reduces activity loss and prevents contamination. Thus, strict control of temperature and application of mechanical stress are necessary. Cell lysis due to mechanical stress will be avoided by the use of carefully selected reactors, pumps, and separation columns. Temperature control is the most important consideration

<sup>18</sup> Ah-Moye D et al. Clinical Biochemistry: Metabolic and Clinical Aspects. London: Churchill Livingstone; c2014. Chapter 26, Introduction to haematology and transfusion science; p. 497–514.

<sup>19</sup> Siegel DL. Re: Costing of ECO-RBC units [Internet]. Message to: Carly Catella. 2018 Apr 3, 10:00 pm [cited 2019 Apr 15]. [about 6 paragraphs].

for the handling of RBC units in this process. Hospitals typically adhere to the “thirty-minute” and “four-hour” rules stipulating that RBC units should not be reissued after thirty minutes of room temperature exposure, and that transfusions should be completed within four hours. The risks associated with exposure to temperatures above 4°C include bacteria proliferation and loss of activity.<sup>20</sup> However, studies have shown that bacteria proliferation is insignificant for times less than two hours, and loss of activity is negligible.<sup>21</sup> The enzymatic treatment process must maintain proper temperature control throughout, and have a total time below two hours at a temperature above 4°C. Sterilization processes will be implemented to reduce the risk of contamination and quality control testing will be performed prior to the distribution of the treated RBC units to ensure that there is no measurable loss in activity or bacterial contamination.

Customers also require consistent packaging and delivery for efficient use in hospitals. The most important considerations in this area are meeting FDA packaging guidelines, salient labeling, and compatibility with existing storage options. The FDA requires that blood storage bags be sterile and clear to allow for observation of hemolysis or change in color. They must also be clearly labeled with antigen type and may be color coded.<sup>22</sup> Delivery must be prompt to maximize the usable shelf life of the RBC unit, which is capped at 42 days in most situations<sup>10</sup>. Measures will be put in place to ensure a quick turnaround time for the treatment process to allow the customer to have relatively fresh RBC units.

### 4.3. Regulatory Requirements

The use of enzymatically converted RBC units in the place of traditional RBCs will require approval from the FDA. Due to the extensively processed nature of this product and its differentiation from traditional RBC products, it will need to undergo comprehensive clinical testing. A Biologic License Application (BLA) for ECO-RBCs will need to be approved.<sup>13</sup> The FDA will look for good manufacturing practices associated with the large scale production and proper quality control of the enzymatically converted RBCs in their approval process. Additional concerns arise from the novel nature of the enzymatically converted RBCs, which include potential

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<sup>20</sup> Ramirez-Arcos S et al. Evaluating the 4-hour and 30-minute rules: effects of room temperature exposure on red blood cell quality and bacterial growth. *Transfusion* 2013; 53(4): 851-859.

<sup>21</sup> Vosta J. FDA Evaluation of Red Cell Products. Laboratory of Cellular Hematology, U.S. Food and Drug Administration, 2016.

<sup>22</sup> Diamond SL. Re: Cost of FDA Approval of ECO-RBCs [Internet]. Message to: Carly Catella. 2018 Apr 2, 11:08 am [cited 2018 Apr 15]. [about 44 paragraphs].

toxicity and efficacy issues. Studies will need to be performed to confirm that “immunogenicity, reduced cell flexibility, increased fragility, low oxygen delivery capacity, and unanticipated toxicities” are not properties of the enzymatically converted RBCs.<sup>4</sup> *In vitro* studies will need to be performed to study the morphology, biochemistry, and the hemolysis of the enzymatically converted RBCs. Phase I and II clinical trials must be performed to assess kinetics, and Phase III clinical trials need to be performed to assess safety and efficacy of the enzymatically converted RBCs.

The design and performance of clinical trials for FDA approval are out of the scope of this project; however, it is important to consider what these trials would encompass. Clinical trials for the enzymatic conversion using ECO-B have already shown the feasibility of the process, but more rigorous studies inclusive of enzymatic conversion with ECO-A need to be performed<sup>14</sup>. These studies would primarily look for safety and efficacy markers that match or surpass those of RBC units collected via apheresis. Safety studies would address the immunogenicity of the enzymatically converted RBCs by looking for antibody formation. The efficacy markers would include markers for hemolysis, hemoglobin concentration, and oxygen dissociation. Phase III studies would address both safety and efficacy concerns, and additional Phase IV post market safety studies would likely be necessary.<sup>4</sup> Safety concerns would be addressed by testing for mismatched immune response and the safety of the potentially augmented clearance pathway. Phase III and IV studies would compare the efficacy of ECO-RBCs to that of wild-type RBCs. They would also compare and contrast the circulatory half-life, hemoglobin clearance, and storage stability of both wild-type and enzymatically converted RBCs.

While Phase I clinical trials can be performed in healthy patients to test for safety and dose escalation, Phase II and III clinical trials must be done in patients with measurable indications of need. These indications can range from bleeding from elective surgery to traumatic bleeding. However, indications of need like traumatic bleeding are difficult criteria under which to enroll patients because obtaining informed consent is challenging and the transfusion of the RBCs is difficult to control<sup>12</sup>. Indications like anemia and bleeding from elective surgery are easier indications as researchers have time to obtain informed consent prior to transfusion, and the response to wild-type RBCs is well characterized, as both of these indications routinely utilize RBC transfusions. Clinical trials can be performed for either or both of these indications; for the

purposes of this report, the specific design of the clinical trials is not considered. The expected cost to complete these trials is projected to be \$100-200 million.<sup>22</sup>

#### **4.4. Summarized Project Description**

The design described in the project consists of two separate processes: (1) the production of the necessary bacterial glycosidases for the RBC conversion and (2) the RBC treatment process. The production of bacterial glycosidases was determined to be done in-house rather than purchasing the enzymes in bulk to reduce costs. Additionally, the implementation of this portion of the design gives greater control over the *E. coli* strains which are used for upstream processing. By establishing the upstream process design, if further research finds more suitable bacterial glycosidases or enzymes that can perform the same function, the entire plant design will have already been established. The only change required is the substitution of the cell line used for enzyme production.

The upstream processing consists of three growth phases: (1) inoculum grow up, (2) microbial fermentation in a seed reactor, and (3) production fermentation in two large stainless steel fermenters. This production train was chosen to prevent overdiluting the cell samples and accidentally inducing cell death. Additionally, this setup allows for high-cell density microbial cultures, which is required since the enzyme product yield is still relatively low.

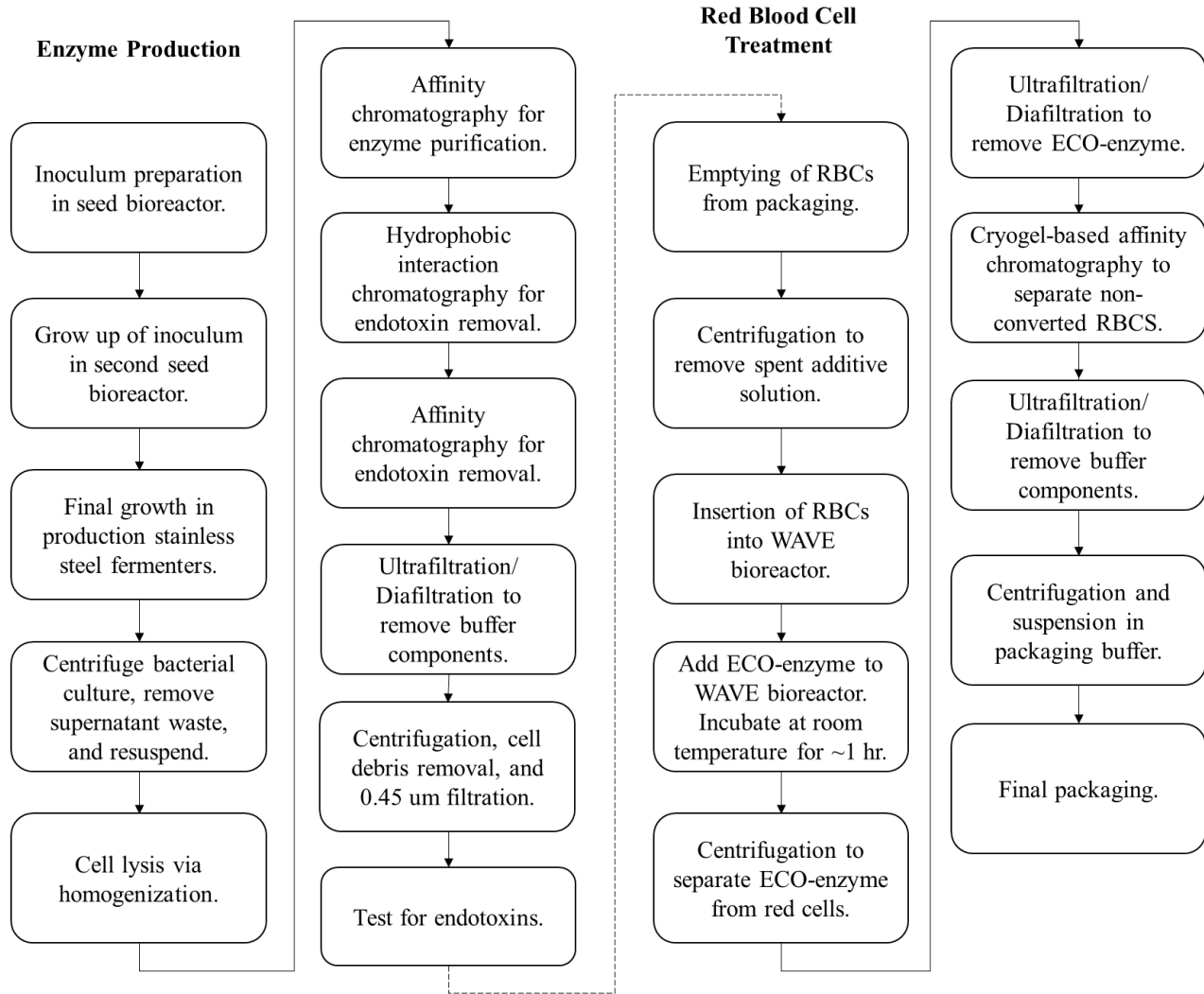
The downstream purification of the enzyme products involves numerous chromatography and filtration steps to reduce endotoxin levels below the limitations established by the FDA. Since the enzyme is cytoplasmic, cell membrane disruption is done using microfluidization to harvest the internalized enzyme. Then the product solution undergoes three chromatography steps: (1) cobalt ion affinity chromatography to remove cell debris and purify the enzymes, (2) hydrophobic interaction chromatography to separate endotoxins, and (3) endotoxin affinity chromatography to further separate endotoxins that may have unexpected hydrophobicity values. The product solution will then undergo ultrafiltration/diafiltration using a tangential flow filtration system to remove buffer components. Lastly, the product solution will undergo two bulk microfiltration passes to further eliminate endotoxins and concentrate each enzyme.

The RBC treatment process is simple, requiring that the donor RBCs be charged into a WAVE bioreactor to which ECO-zyme will be added. The RBCs will be agitated with the enzyme



for one hour at 25°C and at a pH of 7.4-7.6. Afterwards they will undergo microfiltration/diafiltration to eliminate the enzyme from the treated RBC suspension, as the presence of enzyme in unpurified samples has been found to show adverse reactions in pre-clinical animal models. Lastly, to guarantee that the treated RBCs will not provoke an immunogenic response in patients, a specialized, cryogel-based affinity chromatography step will be used to separate purely converted RBCs from unconverted ones. This chromatography system uses a soft, polymer gel matrix, instead of traditional resin, that is coated with a protein A and antigen-targeting IgG antibody complex that will bind cells that still have A or B antigens. The soft cryogel was chosen rather than traditional affinity chromatography resin because it is proven to have minimal cell lysis (less than five percent), to release bound cells by applying mechanical stress, and allowing multiple uses of the affinity binding IgG antibody.

The overall process flow diagram in Figure 4.4.1 illustrates the designed production process macroscopically. All final RBC units will undergo numerous quality control tests (flow cytometric analysis and thin layer chromatography (TLC) quantification of surface antigens) to confirm that negligible amounts of non-converted RBCs remain in the transfusions units that will be given to patients.



**Figure 4.4.1.** Process flow diagram for the creation of universal RBC units via enzymatic conversion.

#### 4.5. Facility Location and Requirements

An important decision that determines the feasibility is choosing the location in which the plant design should be executed. Given that the production goal that this project seeks to fulfill depends on hospitals requiring substantial blood supplies, establishing the facility in a metropolitan area with a strong hospital system was deemed a necessity. Furthermore, given the biotechnology nature of the project and the possibility for research collaborations to optimize the RBC treatment process and perhaps explore alternative enzymatic treatments, it was decided that the facility

should be established in a biotechnology hub. The Boston metropolitan area fits all of these considerations, with significantly cheaper utilities pricing and space rental costs compared to the biotechnology hub in California. It also has one of the largest and most well-funded hospital in the United States, Massachusetts General Hospital, which allows for both service partnerships and research opportunities. Additionally, developing this RBC treatment platform in the Boston metropolitan area also allows expansion in additional metropolitan markets easier. There are other major numerous hospital systems along the East Coast relatively close in proximity: New York City, Philadelphia, Newark, among others.

Although many of the customers are expected to be directly in Boston, the new facility for RBC treatment will be built in Medford, MA, which is located less than five miles away from Cambridge, MA. This allows it to be in close proximity to Massachusetts General Hospital and major universities such as Harvard University, Massachusetts Institute of Technology, Tufts University, and more. The rental pricing per square foot of land is also significantly cheaper than in Boston: \$30 versus \$60 per square foot, according to realtor Cummings Properties.

The rental space will come equipped with biosafety cabinet hoods for conducting sterile cell culture and RBC management (if necessary). It also contains clean room spaces that can be used for RBC handling and other sterile procedures. Lastly, the facility comes equipped with an autoclave room to sterilize equipment, which will be fundamental in both research and the actual upstream *E. coli* cell culture process, which uses an autoclavable glass bioreactor vessel. The use of this autoclave is included in the facility's rent. The property also features a construction-ready zone with HVAC (heating, ventilation, and air conditioning) which will be used for sterile air.

Additionally, a vacuum system will be establishing for better biowaste management and to eliminate exposing bloodborne pathogens to the environment. Additionally, Cummings Properties has in-house design and construction teams that can be employed to make necessary modifications before beginning production. This helps outsource plant design while reducing costs. In particular, the design of three 900 ft<sup>2</sup> cold rooms (subject to change depending on factors such as equipment size constraints and personnel maneuverability) will be organized with Cummings Properties and possible third party contractors.

# **Section 5:**

# **CTQ Variables**

## 5. Critical-to-Quality (CTQ) Variables

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### 5.1. Endotoxin Concentrations

Endotoxins are the single most important class of pyrogens, substances that cause fevers when injected into humans and animals. Although fevers induced by endotoxins are not life threatening, endotoxin presence in blood transfusion units can have numerous adverse effects, even potentially fatal consequences. Endotoxins are continuously shed from the outer membrane of viable gram-negative bacteria, such as *E. coli*, and are also released when the bacterial cell dies. In *E. coli*, endotoxins come in the form of complex aggregates of lipopolysaccharides (LPS). The LPS consists of an innermost region composed of hydrophobic fatty acid groups, or lipid A, and a central and outermost region composed of hydrophilic polysaccharides. They may cause uncontrolled activation of mammalian immune systems, releasing a storm of pro-inflammatory agents, such as cytokines and interleukins, which may lead to septic shock in patients. Additionally, this inflammatory reaction may cause either damage to the endothelial layer of blood vessels or vasodilation, both of which eventually lead to leakage from the vessel walls. The substrates required to form a blood clot in the case of injury may be diminished or depleted. Given that blood transfusion units are often used in scenarios in which hemorrhage will occur, patient safety is at risk if the presence of endotoxins prevents proper clotting. Other common consequences of endotoxin contamination are, but not limited to, cardiac malfunction, internal organ failure (usually of the kidneys, adrenal glands, and the lungs), gangrene in the limbs, and hemorrhage-induced Waterhouse-Friderichsen syndrome.<sup>23</sup>

Due to the serious risks associated with endotoxin contamination, the FDA has set limits on concentration of endotoxin for medical devices, parenteral drugs, and any type of ingestible substance for medical treatment. The endotoxin limits currently accepted by the FDA are summarized in Table 5.1. For most medically injected and transfused substances, the limit is set at <0.25 EU/mL, where EU is endotoxin units. One EU equals approximately 0.1 to 0.2 ng endotoxin/mL of solution.<sup>24</sup>

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<sup>23</sup> Raetz CRH and Whitfield C. Lipopolysaccharide endotoxins. Annual Review of Biochemistry 2002 Jul; 71:635-700.

<sup>24</sup> Bacterial Endotoxins/Pyrogens, Inspection Technical Guides. U.S. Food and Drug Administration (2015).

**Table 5.1.** Endotoxin limits for various products, as established by the FDA.

WFI	$\leq 0.25$ EU/mL
LVPs	$\leq 0.5$ EU/mL
Hlb Vaccine	$\leq 50$ EU/mL ( $\leq 25$ EU/dose)
Factor IX	$\leq 0.1$ EU/mL ( $\leq 5$ EU/dose)
Immune Serum Globulin	$\leq 0.91$ EU/mL ( $\leq 5$ EU/dose)

Given that bacterial glycosidases are going to be used to cleave the A and B antigens from the red blood cell surface to convert them to type-O, it is important to minimize the endotoxin concentration in the enzyme solution that is going to be used to treat the RBCs. Any endotoxins remaining in the enzyme solution could also end up in the transfusion units produced. For this reason, three chromatography columns are used to maximize elimination of endotoxins from the final enzyme product. Additionally, thorough quality control tests will be performed on all converted RBC units, using Limulus Amebocyte Lysate (LAL) assays. The LAL kinetic turbidimetric assay<sup>25</sup> will be used to detect endotoxin concentration down to 0.01 EU/mL. These precautionary measures will help prevent adverse effects on patients and eliminate endotoxins prior to treating RBCs. Literature has reported that minimal quantities of endotoxin in the blood can alter RBC morphology and functionality and may even induce hemolysis.

The endotoxin concentration was a heavily considered variable in this process design. We assume that for our product to receive FDA approval, it must satisfy the threshold values established for water-for-injection (WFI), given that the RBCs will be resuspended in preservation solution made from WFI. Even though this project is conceptualized as a start-up and funding is highly dependent on available funding from investors and government subsidies, minimizing endotoxin concentration was deemed a primary concern. While the costs associated with the presence of two chromatography columns dedicated to endotoxin removal are high, they help guarantee that the produced ECO RBCs will have endotoxin values well below FDA standards.

<sup>25</sup> Lonza. Traditional Kinetic Limulus Amebocyte Lysate (LAL) Assay User Quick Guide. Walkersville, MD: Lonza Walkersville; 2014 [cited 2018 Apr 16]. Available from: <https://www.lonza.com/products-services/pharma-biotech/endotoxin-detection/endotoxin-detection-assays/kinetic-turbidimetric-lal-assays.aspx>

## 5.2. Final Universal RBC Product Purity

This project assumes that the bacterial glycosidases,  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase, needed to convert type-A and type-B RBCs to type-O manage to convert >90% of the treated RBC population. However, this assumption implies that there are still cells with antigens that could cause immunogenic responses if transfused into incompatible patients. Given that this project seeks to provide a product and service that can eliminate the concern of blood transfusion incompatibility, a specialized chromatography column was designed for the sole purpose of separating non-converted RBCs from successfully converted ones. From an ethical standpoint, this imperfect conversion could not be ignored as it could lead to health problems and potentially have fatal consequences in patients. Even though this additional chromatography column adds significant additional operation and installation costs, its inclusion helps guarantee patient safety, comply with ethical concerns, and provides a proper business model that future venture capital investors may appreciate.

## 5.3. Production Capacity

The most important variable in this project was the production capacity of this small-scale RBC treatment plant. Given the limited size and finances of the project, the production goal for enzymatically converted RBCs is used to determine all other key parameters in this process: annual enzyme mass required, number of donor transfusion units that need to be treated to satisfy production goal, physical and financial constraints to place on process design, sizing parameters, among others. As a result, a realistic production goal of 200,000 transfusion units of enzymatically converted RBCs was established. This production goal would satisfy approximately 25% of the annual RBC transfusion unit demand required by a major metropolitan hospital. In a broader perspective, this would satisfy nearly 1% of the annual blood demand in the United States.

Even though the production goal is realistic for the start-up scale of this project, it still requires a relatively high cell density bacterial culture to produce sufficient enzyme to satisfy production. The genetically engineered *E. coli* BL21(DE3) cells that produce the  $\alpha$ -galactosidase

and  $\alpha$ -N-acetylgalactosaminidase enzymes have relatively low protein yield coefficients.<sup>26</sup> To minimize costs of purchasing more equipment and maximize return on investment, the cell density was increased from a common 16 grams of biomass per liter of culture to 29 grams per liter. This operating condition was then used as the second most important variable used in sizing all reactors for upstream and downstream processes.

#### 5.4. RBC Storage Temperature

The optimal storage temperature value for RBCs outside of physiological conditions is 4°C. These storage conditions allows them to remain viable for the entirety of their 42-day shelf life. If cells are kept at higher temperatures, they begin to degrade over time. Transfusion policies have only recently become more stringent in the United States and have not established how long an RBC transfusion unit may remain at room temperature. A common practice established by the Medicines and Healthcare Products Regulatory Agency in the United Kingdom, however, states that RBC transfusion units that have been exposed to an ambient temperature of 25°C for four or more hours should be discarded immediately<sup>27</sup>. Their decision to implement this policy comes from medical reports that found that the oxygen-transfer capability of RBCs is drastically reduced if the cells are not stored at 4°C for more than four hours.

This project assumes that the methodology applied in the United Kingdom should be a standard in transfusion medicine. Thus, the entire RBC treatment process, with the exception of the enzymatic conversion to type-O, is performed at 4°C. As a result, the design schematic presented in this report includes calculations for numerous, large-sized cold rooms to satisfy the strict temperature requirements needed to maintain RBC viability.

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<sup>26</sup> Sivashanmugam A et al. Practical protocols for production of very high yields of recombinant proteins using *Escherichia coli*. Protein Science 2009; 18: 936-948.

<sup>27</sup> Brunskill S et al. What is the maximum time that a unit of red blood cells can be safely left out of controlled temperature storage? Transfus. Med. Rev. 2012; 26(3): 209-223.



# **Section 6:**

# **Product Concepts**

## **6. Product Concepts**

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Not applicable.

# **Section 7:**

# **Superior Product Concepts**

## **7. Superior Product Concepts**

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Not applicable.

# **Section 8:**

# **Competitive/Patent Analysis**

## **8. Competitive/Patent Analysis**

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Not applicable.

# **Section 9:**

# **Preliminary Process Synthesis**

## 9. Preliminary Process Synthesis

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### 9.1. Initial Considerations

Prior to upstream production, *Escherichia coli* strains that produce  $\alpha$ -N-acetylgalactosaminidase or  $\alpha$ -galactosidase need to be genetically engineered. These two enzymes were originally discovered in *Elizabethkingia meningosepticum* and *Streptomyces avermitilis*, respectively.<sup>4</sup> For the purposes of this design, it was decided that the best approach for ensuring high protein yielding bacterial cells are used in the fermentation process is to contract the preparation of the *E. coli* cell lines to a contract research organization. Many of these organizations have well-established gene editing platforms that can engineer a specific cell line in four to six weeks. This will allow plant setup to occur simultaneous with cell line preparation. Additionally, it eliminates expenses required to initially perfect and optimize an internal gene editing platform to prepare the desired *E. coli* strains. Given the limited funds allotted to this project due its start-up nature, it was determined that expediting the production stage should be prioritized over the development of an internal gene editing system. Future sales funds can be allocated to research once the project is established and is able to deliver functional universal blood cells.

The preparation of these bacterial cell lines will be contracted to GenScript, a contract research organization that specializes in genetic engineering using the CRISPR-gene editing system. The product of each cell line will cost \$5,400. GenScript was chosen for their  $\lambda$  Red-CRISPR/Cas9 gene editing platform, which uses a two vector system that enables editing accuracy down to a single base pair. It is a seamless platform with high efficiency (>95%) and easy precision.

The entire coding region of *Elizabethkingia meningosepticum*  $\alpha$ -N-acetylgalactosaminidase, shown in Figure 9.1.1, will amplified by polymerase chain reaction (PCR) from genomic DNA with the following primers<sup>4</sup>:

1. FLAV28: 5'-GCGGGATCCAATGGGTGCTTTAATTCCTCGAGC-3'
2. FLAV13: 5'-CGGCGGCCGCTTAGTAGTCGTCATTTATTGCAAATG-3'.

The product will be ligated into the pCR4-Topo TA vector to give a full length FLAV28/13US construct. A truncated construct lacking the N-terminal signal peptide will then be amplified by PCR from the FLAV28/13US construct using the following primers/restriction sites: FLAV34US, 5'-GCGGGATCCGAATTCTAAAAAGGTAAGAATAGCTTTTATAGC-



3'/EcoRI; FLAV13, 5'-CGGCGGCCGCTTAGTAGTCGTCATTTATTGCAAATG-3'/NotI. The PCR product will be inserted into EcoRI/NotI digested pET28 expression vector to create a N-terminal truncated construct with a histidine-6 (His<sub>6</sub>) tag at the N-terminus to be used for future separations.<sup>4</sup>

```

1  atggggcgct taattccctc gagcacttta ttcaacattt tcgatttcaa ccctaaaaag
61  gtaagaatag cttttatcgc tgttggttta cgtggacaaa ctcacgtaga aaatatggca
121 agacgtgatg atgtggagat tgtagcattt gcagatccgg atccttacat ggttggacgt
181 gcacaggaaa ttctgaaaaa gaatggcaag aagcctgcta aagtttttgg aaatggtaat
241 gacgattaca aaaacatgct taaagataaa aatattgatg ctgtttttgt atcatctcca
301 tgggaatggc accacgaaca tggcgtagca gctatgaaag ctggtaaaat tgtcggaaatg
361 gaagtttccg gtgctataac actggaggaa tgttgggatt acgtaaaagt atctgaacaa
421 accggagttc cgtaaatggc attagaaaat gtatgctaca gacgcgatgt aatggctatc
481 ctgaatatgg taagaaaagg aatgttcgga gaacttggtc acggaacagg aggtaccag
541 cacgatttaa gaccagtttt attcaacagt ggcatcaatg gtaaaaacgg agatgggtgt
601 gaattcggag aaaaagcatt tagtgaagcc aagtggagaa cgaaccacta taaaaacaga
661 aacggggaac tttaccctac tcatggtgtt ggtccattgc atacaatgat ggatattaac
721 cgtggaaaca gattactaag attatcatct tttgcatcca aagcaagagg attacataaa
781 tacatcgtgg ataaagggtg agaaagccat cctaatacaa aagtagaatg gaaacaagga
841 gatattgtta ccaactcagat ccagtgtcac aacggagaaa ctattgtatt aacacacgat
901 accagcttac aaagaccata taacttagga ttcaaagttc aaggtagaga aggtcttttg
961 gaagatttcg gctggggaga agcagcacia ggatttattt acttcgagaa gattatgaac
1021 cattctcaca gatgggatag ttctgaaaaa tggattaaag aatatgatca ccctatgtgg
1081 aagaagcatg agcagaaaagc tgttgggtgc ggtcatggcg gtatggatta cttcttagat
1141 aatacgttcg tagaatgtat taaaagaaat gaagcattcc cgttagatgt ctatgatctg
1201 gctacatggt attccattac tcctcttagt gaaaagtcta tcgctgaaaa cggtgccgtt
1261 caggaaattc ctgattttac aaacggtaaa tggaagaatg ctaaaaatac atttgcaata
1321 aatgacgact actaa

```

**Figure 9.1.1.** The original gene sequence that encodes  $\alpha$ -N-acetylgalactosaminidase (GH109), which cleaves A antigens on the surface of RBCs.. This enzyme was originally discovered in *Elizabethkingia meningoseptica*. This sequence will be engineered into a plasmid vector that also encodes genes for kanamycin and chloramphenicol antibiotic resistance. The engineered plasmid will be knocked-in to *E. coli* using  $\lambda$  Red-CRISPR/Cas9 gene editing to develop a bacterial platform that can be selected using antibiotic resistance to mass produce this enzyme.

The engineering of the  $\alpha$ -galactosidase-producing strain of *E. coli*, originally from *Streptomyces avermitilis*, will undergo a near identical gene editing process to also insert His<sub>6</sub> tags as previously described. The original gene sequence for  $\alpha$ -galactosidase is shown in Figure 9.1.2.

```

1  atggctcacg gatgctccgg aggggctatg tctcggttcg tgtttcttgg ggtggcactc
61  gcgttgctcg gaggtgccac cagtcctgcc gccgcggcgc cgagggtgac gccggtggtg
121 gtcgacgtgg acgactacgg agccgacccc accggcggga cggactcgac gcccgctgtc
181 gccgcggcgc tcgggcacgc caagagcgtc gaccggcggg tcaggatcgt cttctccaag
241 ggcacctacc agctgtaccc cgagcggggc gagacgcgtg agctgtatat gtccaacacc
301 gtcggcgccc accagcgtaa ccggggacaag aagatcgggc tgctcgtcga ggacatgcac
361 gacgtcaccc tcgacggcgg cgggggccaag ctcgtccacc acggtcttca gacggcggtt
421 gcgtcgatcc gctcgaccga tgtgaccttc cagaacttct ctttcgacta cgcggccccc
481 gaggtcatcg acgcgaccgt gccaccacc ggtgtcaccc acggacacgc ctaccgcgtt
541 ctgaagatcc ccgccggcag cccgtaccgg gtgaacggca cgcacatcac ctggctcggc
601 gagaccagtc ccgccaccgg gcagccgtac tggtcgggtg tcgacgggct ccagtaacac
661 cagatccacg accccgaggc gcaacggacc tggcgcggag acaaccccct gttcaacgac
721 gtcgcgcggg tcaccgacct cggcggcggc cggatccgga tcgactacac caccgcggcg
781 cggccggcgg acgcggggct cgtctaccag atgcgcctca tcgagcgaac ggagccgggc
841 gctttcatct gggagtccaa gaacgtcacg atgcgctcga tgaacgccta ctacctacg
901 tccttcggcg tggtagggga gttcagcgag aacatctcca tcgacaaggt gaacttcgca
961 cccgatccga ggtccggcgg gtcgacggcc tcgttcgagg acttcgtgca gatgtccggc
1021 gtcaaggagg aggtctcgat caccgggagc ctcttcgacg gcccgcacga cgaccgatc
1081 aacatccacg gcacctacct cgaggctcgtc gggaagccgg ggccgtccac cctcaccctc
1141 gcctacaagc acccgagac cgcgggattc ccacagttcg ccccggtga cgaggctgag
1201 ttcgccacca agcgcacgat gaccccgctg gcggacgcgc acgcacaggt caccgcggtc
1261 gacgggcccga gcggcatgga ccacacgaaa ccgctgacca cgatgaccgt caccttcgac
1321 cggccgggtt ccgccggggt cgagaccggc ggacgggtcg tcgagaacat cacggccacc
1381 ccgtcgggtc tcatctccgg caacgtgttc cgcaacgtgc cgaccgcgg catcctggtc
1441 accacccgca agcccgtcct catcacggc aaccggttcg acgggatgtc catggccagc
1501 atctacgtct ccgccgacgc ctaccagtgg tacgagtccg ggccggtcgc cgacctcacg
1561 atccgcggga actccttcac gcggcccagt ggtccggtca tcttcgtgga gccgaccaat
1621 caggatcatc acccgcgac cccggtgcat cacaacatct cggtcgagca caactcgtt
1681 gacatcgggt acgtcacctg cgtcaacgcc aagagcgtcg gcgggttcgc tttaccggc
1741 aacacgggtc ggcggtgga cggagcggat caccgccgt acacctcacc gctgttcgtc
1801 ttccacggca gttcaggcat ccggtcgcga cgcaaccact acgacaaggg cctgaacacc
1861 tccgtggtca cggactag

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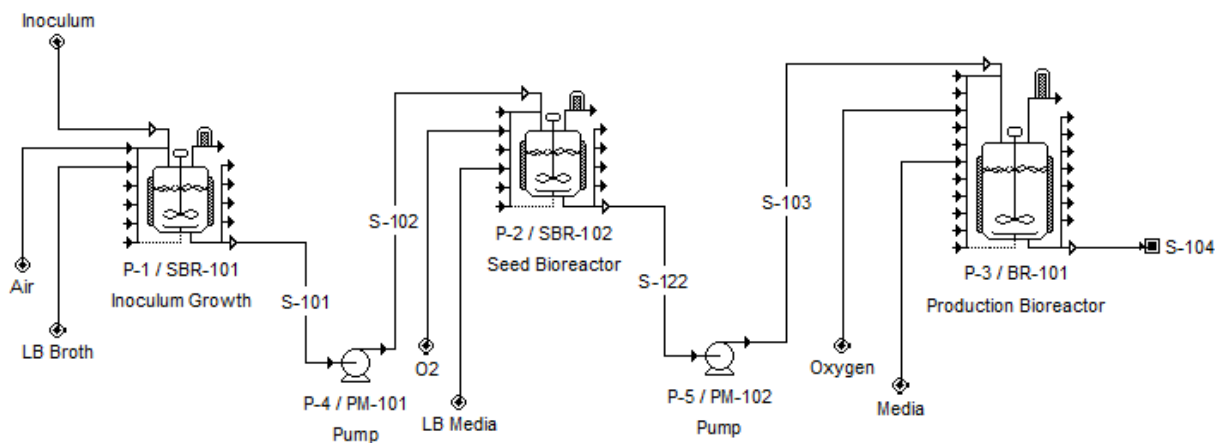
**Figure 9.1.2.** The gene sequence that encodes  $\alpha$ -galactosidase (GH110), the enzyme that is responsible for cleaving B antigens. This enzyme was originally discovered in *Streptomyces avermitilis*. This sequence will also be engineered into a plasmid vector that also encodes genes for kanamycin and chloramphenicol antibiotic resistance.

## 9.2. Upstream *E. coli* Cell Culture Process

### 9.2.1. Reactor and Cell Density Considerations

The final iteration in determining the adequate equipment for culturing *E. coli* cells required analyzing oxygen mass transfer limitations in the design setup. Originally, the system was designed exclusively using single-use, disposable bioreactor systems (Figure 9.2.1), which are becoming increasingly used in the biotechnology industry. However, single-use bioreactor systems

have difficulty at operating above a volumetric oxygen transfer coefficient  $k_L a$  of  $40 \text{ hr}^{-1}$ . This low value also has the tendency to fall below  $20 \text{ hr}^{-1}$  during operation. Jeffrey Cohen, Ph.D., from Johnson & Johnson expressed concerns that this system would not be able to supply sufficient oxygen to the cell culture and could result in massive cell death.



**Figure 9.2.1.** The original schematic used in the initial conceptualization of the project. The original design consisted of using three single-use bioreactors, with working volumes of 0.110 L, 5.44 L, and 200 L, respectively. The production bioreactor was set to achieve a maximum cell density of 100 grams per liter, as certain high microbial density fermentation protocols claim. The concept of using a single-use production bioreactor because it has limited oxygen transfer capabilities and is unable to satisfactorily support high-density microbial cultures.

Many aggressive microbial cultures, however, require systems that can produce a volumetric oxygen transfer coefficient greater than  $360 \text{ hr}^{-1}$  to satisfy the oxygen demand of fairly high density bacterial cultures.<sup>28</sup> Another complication associated with using single-use systems is that these high volumetric oxygen transfer coefficients often require gas flows of two vessel volumes per minute. Satisfying this gas flow demands is made difficult since single-use systems have constrained gas flow rates due to pressure safety limits of the single-use flexible container. The required low operating backpressure decreases the gas flow capacity of exhaust filters. For

<sup>28</sup> Sartorius Stedim Biotech GmbH. High-cell-density Cultivation of Escherichia coli in a BIOSTAT® D-DCU 10-3 Stainless Steel Bioreactor [Internet]. High-cell-density Cultivation of Escherichia coli in a BIOSTAT® D-DCU 10-3 Stainless Steel Bioreactor. Goettingen, Germany: Sartorius Stedim Biotech GmbH; 2013 [cited 2018 Apr 17]. Available from: [https://www.sartorius.com/mediafile/AppI\\_BIOSTAT\\_D-DCU\\_High-cell-density-Cultivation\\_E\\_Coli\\_SBT1015-e.pdf](https://www.sartorius.com/mediafile/AppI_BIOSTAT_D-DCU_High-cell-density-Cultivation_E_Coli_SBT1015-e.pdf)

these reasons, the use of single-use bioreactors were discarded for the purposes of our microbial fermentation.

Recent progress has been made in developing single-use fermenters that vastly outperform single-use bioreactors, potentially providing a solution to the lack of single-use technology for bacterial growth. During equipment selection, however, it was noticed that there are major discrepancies among different sources on the actual volumetric mass transfer coefficient. Some reports stated that the single-use fermenters could operate achieve a  $k_La$  of  $80 \text{ hr}^{-1}$ , while others stated that it could be well above 400. Additionally, the experiments from which this data was obtained often reported that there was difficulty in controlling cooling capacity and foaming levels, both of which could cause enzyme denaturation upon protein expression. These considerations led us to conclude that a stainless steel fermenter, which has well-studied volumetric oxygen transfer coefficients and agitation configurations, would be a safer choice for conducting all cell growth. Additionally, stainless steel reactors can be seen as permanent investments with no further major operating costs (with the exception of maintenance, CIP, and SIP procedures). Using single-use technology for microbial fermentation though will require continuous purchases of storage bags and containers for the cell harvest, which quickly add on to operating costs.

The final unit chosen to serve as the production bioreactor was the BIOSTAT® D-DCU from Sartorius<sup>28</sup>, which has a volumetric oxygen transfer coefficient of  $308 \text{ hr}^{-1}$ . Additionally, due to sizing limitations of available stainless steel fermenters, two fermenters are required per batch to achieve the desired production goal. Please refer to Section 11 to see the final upstream process configuration. Once this reactor unit was chosen, the maximum allowable cell density for the fermentation process was calculated (see Appendix F) to be approximately 29 grams of cell per liter of culture. These renewed calculations after taking into account the oxygen mass transfer limitations of the reactor also helped resolve the concern on the high density at which the reactors originally planned to reach (100 g cells/L). Many were considered that this cell density would be too viscous and prevent the culture from being well mixed, which would have further hindered oxygen availability to the cells.

### 9.2.2. Batch Timing

The original process setup applied cell growth parameters that are significantly different from those more commonly seen in industrial fermentation. The growth rate of *E. coli* cells that

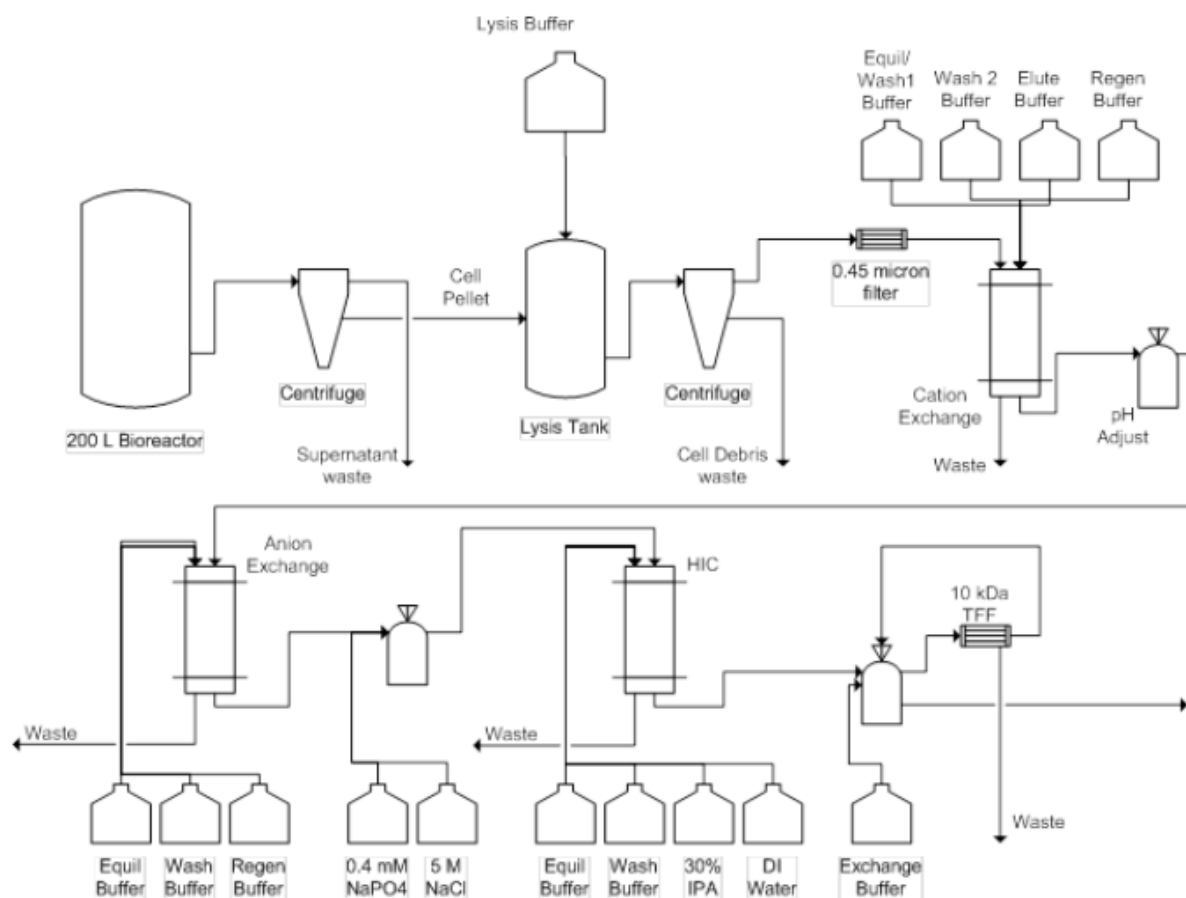
was originally used for the mathematical modeling of cell growth was  $0.60 \text{ hr}^{-1}$ . This value was obtained from several online literature sources and from several pilot plant studies that used *E. coli* to produce protein. The maximum cell density established for each culture was 100 grams cells/L, which is considered the highest possible density that can be attained in microbial fermentations. With these two parameters, the calculated batch times were 13.25 hours for the inoculum growth reactor, 5.53 hours for the growth bioreactor, and 8.8 hours for the production bioreactor. Numerous industrial consultants, however, revealed that these values are several fold lower than what is usually observed in practice. Under ideal conditions in which *E. coli* are grown almost exclusively on glucose, a growth rate between  $0.60$  and  $0.70 \text{ hr}^{-1}$  can be observed. In reality, however, the substrate in the culture media changes over time. *E. coli* excrete acetate upon growth on fermentable sugars. The addition of the acetate substrate causes the growth rate to drop to values between  $0.22$  and  $0.28 \text{ hr}^{-1}$ .

Under their recommendation, it was decided that the final process design would use a growth rate of  $0.25 \text{ hr}^{-1}$ , the average of the range in the presence of excreted acetate. Readjusting the growth model with this new parameter, the batch times increased to 16.08, 23.86, and 28.49 hours for the inoculation growth, seed growth, and production units, respectively. Industrial consultants confirmed that these values coincide with typically observed batch times in small-scale biotechnology and pharmaceutical fermentation processes. Additional adjustments were later made to the model (as explained in Section 10.2.1 once reactor limitations were considered), changing the batch times to change to 17.86, 24.05, and 26.08 hours for the inoculation growth, seed growth, and production units, respectively. These values are still in line with industrial processes, confirming that the material balances for the *E. coli* growth

### **9.3. Downstream Enzyme Purification**

As discussed in Section 5.1, the presence of endotoxins in the enzyme product could have numerous adverse effects. Not only have endotoxins found to affect RBC functionality and viability, but their presence in the enzyme product that is used to treat RBCs may also cause endotoxins to end up in the final packaged RBCs. To minimize the endotoxin concentration in the packaged universal RBCs, the downstream purification process underwent several iterations to explore different chromatography column uses.

Patents recommended a hypothetical separation model that used a cation exchange, anion exchange, and hydrophobic interaction chromatography train to purify  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase from endotoxins<sup>4,10</sup>. The original process flow diagram for the above configuration is shown in Figure 9.3.1. However, a review of the biochemical properties, specifically the electrical charge of both enzymes and endotoxins, revealed that all of these proteins have a high net negative surface charge. Based on this finding, the notion of using any form of ion exchange was discarded since the charge differences among the endotoxins and enzymes was too little to exploit for a separation process. Another consideration was the possible use of size-exclusion chromatography, but this idea was quickly discarded as the molecular weights of  $\alpha$ -galactosidase,  $\alpha$ -N-acetylgalactosaminidase, and endotoxins are roughly 45, 75, and 10-100 kDa, respectively.<sup>4</sup> After consulting with professional engineers, it was deemed that the size differences were too small (less than ten-fold) to be useful for size-based separations.

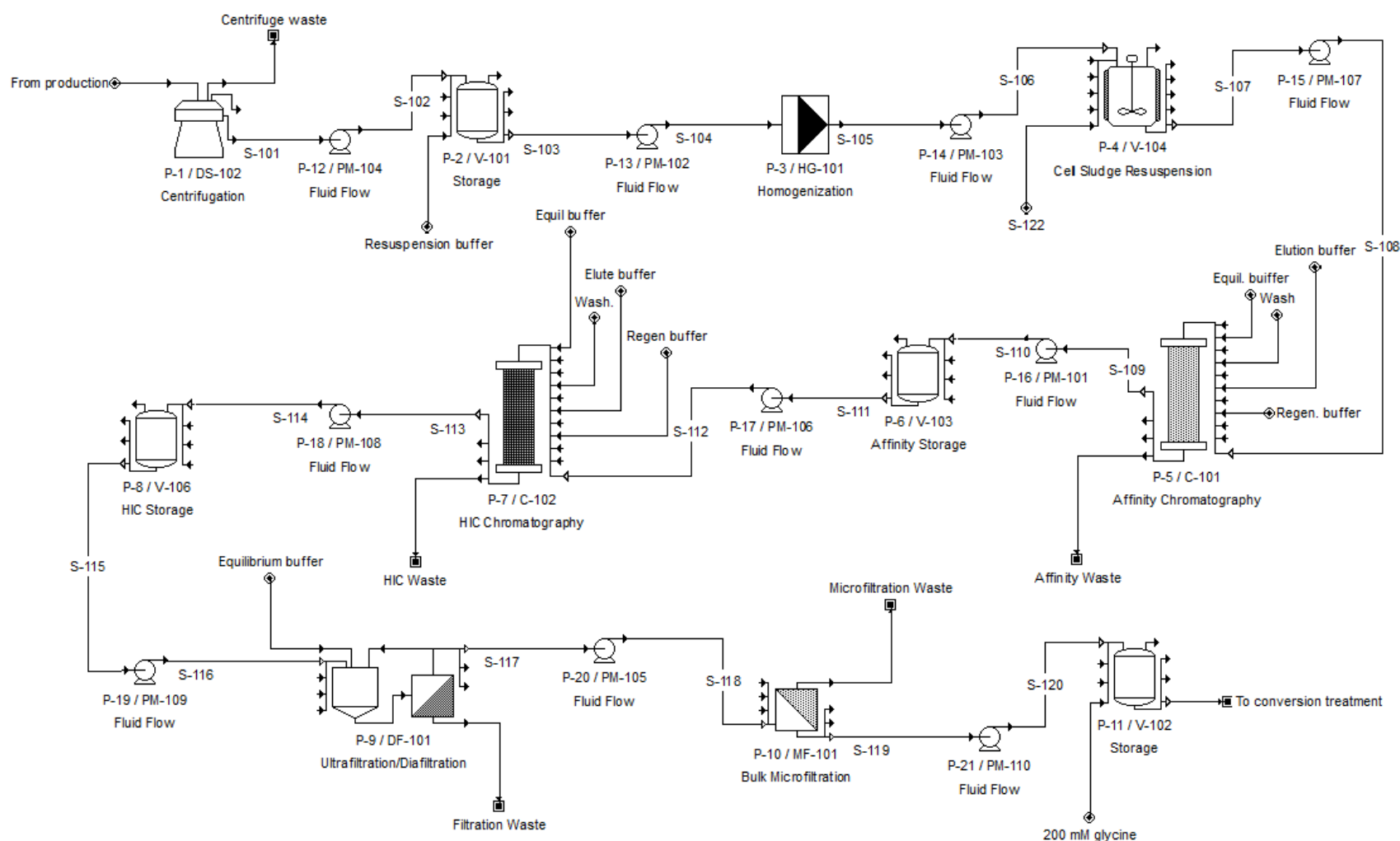


**Figure 9.3.1.** Proposed process flow diagram from patents that explored the hypothetical feasibility of scaling up enzymatic treatment of RBCs to create a universal blood supply.

The last option explored was using an affinity chromatography column that would specifically bind endotoxins using porous cellulose beads coated with poly( $\epsilon$ -lysine) as the resin. Poly( $\epsilon$ -lysine) strongly binds endotoxins with relatively high specificity. Given that this resin does not have the issues associated with surface charge and size that renders the other chromatography techniques inapplicable for this particular separation, an endotoxin affinity chromatography column was incorporated into the process design. The hydrophobic interaction chromatography column was also kept in the design as an additional method to further eliminate endotoxins. Jeffrey Cohen (Johnson & Johnson), Daniel Green (GlaxoKlineSmith), and Robert Luo (GlaxoKlineSmith) all recommended that both chromatography columns be included to ensure that endotoxins were eliminated from the final product as much as possible. Given that the overall goal is to produce universal RBCs that will be transfused into patients, safety and risk minimization should be prioritized. This setup is shown in Figure 9.3.2.

In addition to the chromatography columns meant for separating the  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase enzyme products, it was further recommended that an additional chromatography column be incorporated into the design process prior to the other two to eliminate organelles and cytoplasmic material from the bacterial lysis. Thus, an additional affinity chromatography unit was added at the beginning of the separations process to bind all His<sub>6</sub>-tagged enzyme.

The final iteration of the downstream purification design for  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase took into account in which order the endotoxin affinity and hydrophobic interaction chromatography columns should be placed. This decision was made by performing a financial analysis on the resin required for different setup configurations. It should be noted that although there are endotoxin losses associated with transport losses, we assume that no endotoxin is lost for the design of the chromatography columns. This allows us to have a stricter design that can treat more endotoxin than what may actually be present in the final enzyme product solution. The first step in the separations process involves treating 11.5 grams of LPS endotoxin. Assuming a conservative 90% elimination of endotoxins, the second step must process 1.15 grams of LPS. Table 9.3.1 shows an initial general assessment of reagent costs if the endotoxin affinity column is placed before the hydrophobic interaction column.



**Figure 9.3.1.** Second major iteration of the downstream purification process of ECO-zymes. In this setup, endotoxin affinity chromatography and hydrophobic interaction (HIC) chromatography are used to eliminate endotoxins from the final enzyme product. The elimination of endotoxins is necessary to ensure endotoxin concentrations are low enough so that transfused ECO RBCs do not cause adverse immune responses.



**Table 9.3.1.** Preliminary reagent costing for a process design in which a separations train consists of an endotoxin affinity chromatography column followed by a hydrophobic interaction chromatography column for the removal of endotoxins. The resins in questions are highlighted in light red.

Raw Material	Annual Amount Required		Cost of Raw Material		Cost per RBC Unit	Annual Cost
AGE	2.2	L	\$ 173.00	\$/L	\$ 0.0019	\$ 381.81
Ammonium sulfate	5017.6	g	\$ 51.76	\$/kg	\$ 0.0013	\$ 259.71
APS	285.6	g	\$ 78.60	\$/kg	\$ 0.0001	\$ 22.45
Detergent	431856.3	g	\$ 62.60	\$/kg	\$ 0.1352	\$ 27,034.21
DMAAm	16.4	L	\$ 93.00	\$/L	\$ 0.0076	\$ 1,521.26
Ethanol	4154.3	L	\$ 4.18	\$/L	\$ 0.0868	\$ 17,359.76
Ethylenediamine	78175.7	g	\$ 73.50	\$/L	\$ 0.0320	\$ 6,391.45
Glutaraldehyde	129.8	L	\$ 99.50	\$/L	\$ 0.0646	\$ 12,917.37
Guanadine HCl	48563.2	L	\$ 246.75	\$/L	\$ 6.9758	\$ 1,395,162.01
HEPES	66823.8	g	\$ 223.00	\$/kg	\$ 0.0745	\$ 14,901.70
IgG	1298.2	mg	\$ 790.00	\$/mg	\$ 5.1280	\$ 1,025,600.36
Imidazole	312256.0	g	\$ 265.00	\$/kg	\$ 0.4137	\$ 82,747.84
MBAAm	5712.2	g	\$ 1,044.00	\$/kg	\$ 0.0298	\$ 5,963.54
MES	105392.0	g	\$ 1,003.00	\$/kg	\$ 0.5285	\$ 105,708.18
Na <sub>2</sub> CO <sub>3</sub>	55044.9	g	\$ 137.00	\$/kg	\$ 0.0377	\$ 7,541.15
NaBH <sub>4</sub>	9822.4	g	\$ 118.18	\$/kg	\$ 0.0058	\$ 1,160.76
NaCl	2838618.0	g	\$ 10.38	\$/kg	\$ 0.1473	\$ 29,464.85
NaOH	1113125.4	g	\$ 36.00	\$/kg	\$ 0.2004	\$ 40,072.52
Protein A	4154.3	g	\$ 366.00	\$/g	\$ 7.6024	\$ 1,520,484.99
Sodium phosphate	579760.4	g	\$ 42.00	\$/kg	\$ 0.1217	\$ 24,349.93
TEMED	246.7	mL	\$ 168.00	\$/L	\$ 0.0002	\$ 41.44
Urea	2146439.7	g	\$ 67.40	\$/kg	\$ 0.7234	\$ 144,670.03
HisPur® Cobalt Resin	50.0	L	\$ 4,980.00	\$/L	\$ 1.2450	\$ 249,000.00
Pierce Endotoxin Removal Resin	660.0	L	\$ 20,200.00	\$/L	\$ 66.6600	\$ 13,332,000.00
POROS Benzyl HIC Resin	70.0	L	\$ 3,800.00	\$/L	\$ 1.3300	\$ 266,000.00
Total					\$ 91.55	\$ 18,310,757.32

The costs for the endotoxin affinity resin alone in this configuration reached approximately \$13 million annually (600 L of resin are required annually at a price of \$20,200 per liter). These high costs motivated looking into a new configuration in which the hydrophobic interaction column was placed before the endotoxin affinity column. The general reagent costs of this setup are shown in Table 9.3.2. Altogether the costs of the required volumes of resin between these two columns together only slightly exceeds \$4 million, a three-fold reduction in costs compared to the previous setup. This configuration was thus chosen for the final design.

**Table 9.3.2.** Preliminary reagent costing for a process design in which a separations train consists of a hydrophobic interaction chromatography column followed by an endotoxin affinity chromatography column for the removal of endotoxins. The resins in questions are highlighted in light red.

Raw Material	Annual Amount Required		Cost of Raw Material		Cost per RBC Unit	Annual Cost
AGE	2.2	L	\$ 173.00	\$/L	\$ 0.0019	\$ 381.81
Ammonium sulfate	5017.6	g	\$ 51.76	\$/kg	\$ 0.0013	\$ 259.71
APS	285.6	g	\$ 78.60	\$/kg	\$ 0.0001	\$ 22.45
Detergent	431856.3	g	\$ 62.60	\$/kg	\$ 0.1352	\$ 27,034.21
DMAAm	16.4	L	\$ 93.00	\$/L	\$ 0.0076	\$ 1,521.26
Ethanol	4154.3	L	\$ 4.18	\$/L	\$ 0.0868	\$ 17,359.76
Ethylenediamine	78175.7	g	\$ 73.50	\$/L	\$ 0.0320	\$ 6,391.45
Glutaraldehyde	129.8	L	\$ 99.50	\$/L	\$ 0.0646	\$ 12,917.37
Guanadine HCl	48563.2	L	\$ 246.75	\$/L	\$ 6.9758	\$ 1,395,162.01
HEPES	66823.8	g	\$ 223.00	\$/kg	\$ 0.0745	\$ 14,901.70
IgG	1298.2	mg	\$ 790.00	\$/mg	\$ 5.1280	\$ 1,025,600.36
Imidazole	312256.0	g	\$ 265.00	\$/kg	\$ 0.4137	\$ 82,747.84
MBAAm	5712.2	g	\$ 1,044.00	\$/kg	\$ 0.0298	\$ 5,963.54
MES	105392.0	g	\$ 1,003.00	\$/kg	\$ 0.5285	\$ 105,708.18
Na <sub>2</sub> CO <sub>3</sub>	55044.9	g	\$ 137.00	\$/kg	\$ 0.0377	\$ 7,541.15
NaBH <sub>4</sub>	9822.4	g	\$ 118.18	\$/kg	\$ 0.0058	\$ 1,160.76
NaCl	2838618.0	g	\$ 10.38	\$/kg	\$ 0.1473	\$ 29,464.85
NaOH	1113125.4	g	\$ 36.00	\$/kg	\$ 0.2004	\$ 40,072.52
Protein A	4154.3	g	\$ 366.00	\$/g	\$ 7.6024	\$ 1,520,484.99
Sodium phosphate	579760.4	g	\$ 42.00	\$/kg	\$ 0.1217	\$ 24,349.93
TEMED	246.7	mL	\$ 168.00	\$/L	\$ 0.0002	\$ 41.44
Urea	2146439.7	g	\$ 67.40	\$/kg	\$ 0.7234	\$ 144,670.03
HisPur® Cobalt Resin	50.0	L	\$ 4,980.00	\$/L	\$ 1.2450	\$ 249,000.00
Pierce Endotoxin Removal Resin	80.0	L	\$ 20,200.00	\$/L	\$ 8.0800	\$ 1,616,000.00
POROS Benzyl HIC Resin	650.0	L	\$ 3,800.00	\$/L	\$ 12.3500	\$ 2,470,000.00
Total					\$ 43.99	\$ 8,798,757.32

#### 9.4. Universal RBC Purity and Separations

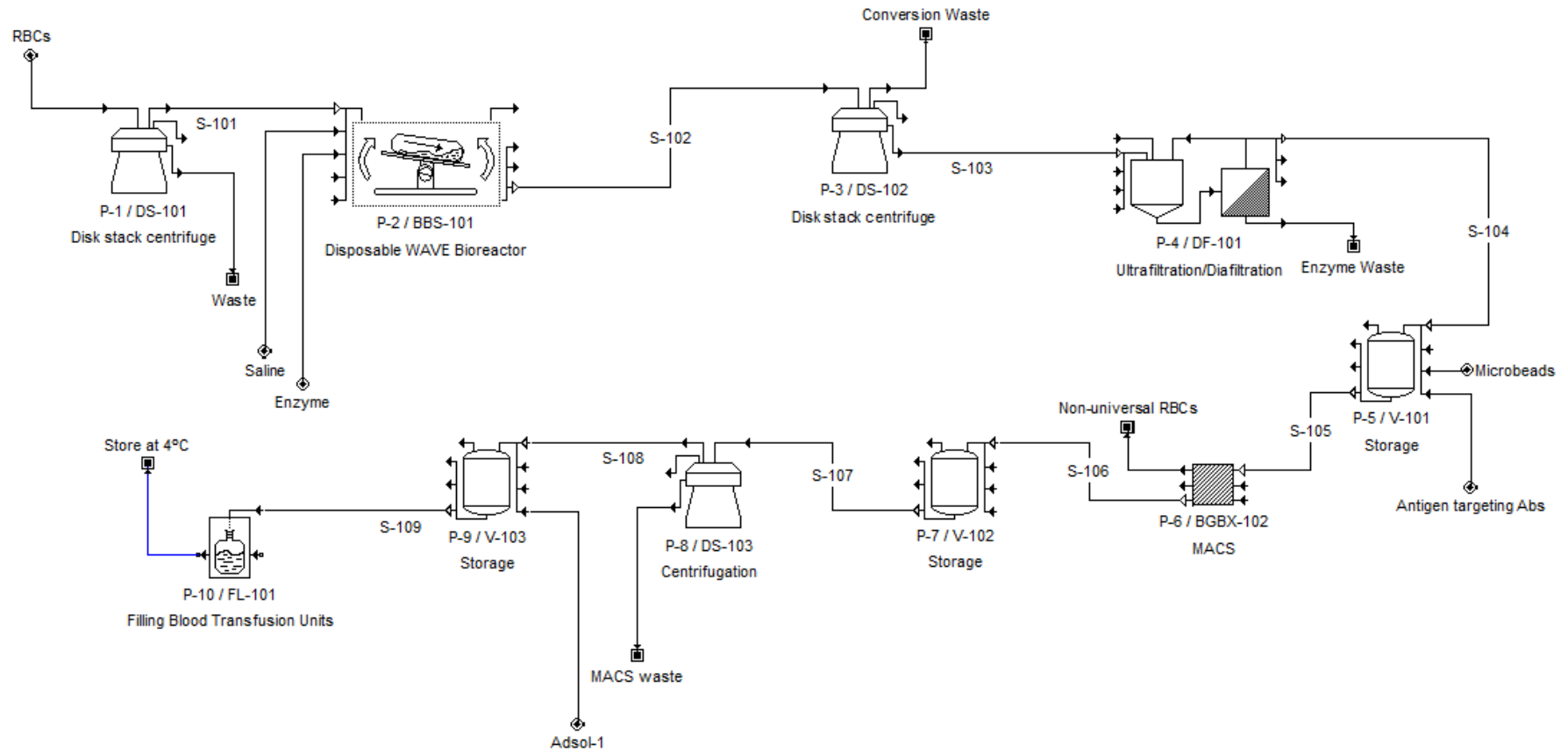
The final major consideration in the process design was the establishment of a unit operation that could separate successfully prepared ECO RBCs from non-converted cells. Originally it was assumed that the enzymes would accomplish a 100% conversion. Upon recommendations from Daniel Green, Ph.D from GlaxoKlineSmith, however, it was decided that this assumption could have potentially severe consequences. Although the kinetics of the bacterial glycosidases have been studied at the bench-scale and shown near complete conversion, there may still be concerns that the scale-up of this procedure may affect the enzymes' contact area with the

RBC, potentially lowering its conversion efficiency. If for any reason that the efficiency is low enough for there to be a substantial presence of non-converted RBCs, then using unseparated RBCs in transfusion units may introduce a high concentration of ABO-incompatible RBCs into the patient. Not only would this be detrimental to one of the main selling points of this service and product, but it could also cause severe health complications in treated patients, raising ethical concerns.

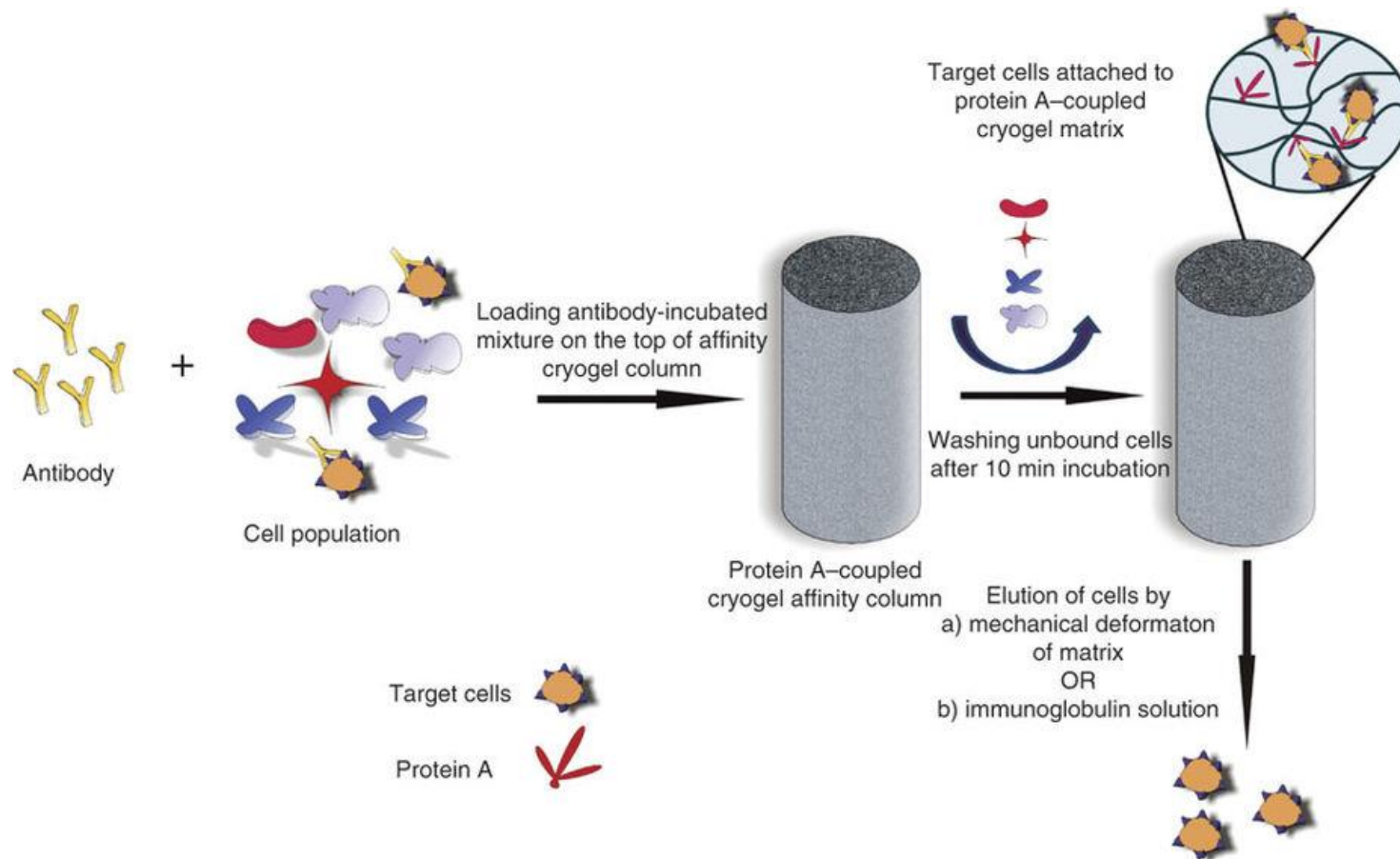
Originally, the process design sought to explore using magnetic-activated cell sorting (MACS) to separate non-converted RBCs, which still possess the A or B antigen, from successfully converted cells (Figure 9.4.1). By using MACS cell depletion separation, non-converted RBCs would be labeled with human blood group antigen-targeting IgG antibodies attached to cellulosic magnetic microbeads. By passing the RBC suspension through a magnetic field, ECO RBCs, which should not be labeled, will pass through while cells with attached microbeads would be magnetically attracted and retained. However, there were two major limitations associated with the use of this technology. First, the microbeads can only label  $10^7$  to  $10^9$  cells. The number of RBCs that each batch will process is on the order of  $10^{15}$ . The second limitation is that the microbeads are only usable once, which would require new antibody and new microbeads for each batch of RBC treatment. The amount of microbeads required, combined with the fact that they are only single-use, would lead to exorbitant costs. For these reasons, this option was discarded after numerous consultations with industrial engineers.

The next iteration of the purification and separation of RBCs replaced the MACS unit operation with a cryogel-based affinity chromatography column. Instead of using a traditional affinity chromatography column, in which its resin is coated in protein A from *Staphylococcus aureus*. Protein A has a strong affinity to the fragment crystallizable (Fc) region of antibodies. In the traditional setup, cells can be labeled with antigen-specific antibodies and passed through the column. Labeled cells will bind to the resin while unlabeled ones will pass through for collection or discarding. These basic principles are illustrated in Figure 9.4.2. A major disadvantage of this setup is the amount of IgG antibody required for annual production, as new labeling antibody will be required for each treatment. Often, the elution buffer used compromises the binding efficiency of the antibody, which can affect later purification attempts. Another major disadvantage is that traditional resins often show significant cell lysis. This last issue could potentially be a significant hindrance in this process design, given that a significant number of RBCs are already lost in each

unit operation. Given that we assume a total 35% loss of RBCs across the entire process, 307,693 RBC transfusion units already have to be requested to provide only 200,000 ECO RBC units in return. Even though a significant number of those original blood bags would be discarded due to no usage before expiration, this process should still attempt to minimize the number of units required for production goals to incentivate hospitals to engage in our service. If lysis is higher though, the 35% assumption may not be valid and more units may be needed, which may push hospitals away.



**Figure 9.4.1.** Simplified process flow diagram of the RBC conversion process in its initial conceptualization stages. While the basic setup from the process flow diagram is used in the final iteration, note that the technique implemented to separate non-converted RBCs is magnetic-activated cell sorting (MACS). It operates by labeling the antigens of non-converted cells with human blood group targeting antibodies. The antibodies are also linked to magnetic microbeads, which can then be attracted to a magnetic field while non-labeled, successfully converted RBCs can pass through to the next unit operation.



**Figure 9.4.2.** Representation of the fundamental principles behind the crygel-based affinity chromatography column. A protein A-coupled cryogen complex will be packed into a chromatography column. RBCs that were not converted still possess their A or B antigens, so they can be labeled with antigen targeting antibodies. This labeled cell population is then introduced into the column and allow to pass. Labeled cells will bind to the protein A coat on the crygel due to IgG antibodies' high affinity to protein A, while all universal RBCs will pass through and be collected. The remaining cells can then be released using mechanical stimulation while the antibodies stay bound for another treatment process.

The cryogel-based method addresses both of these disadvantages and provides unique solutions to each. The binding of protein A and IgG antibodies is typically is very strong, requiring harsh buffers to release bound antibodies and cells in a traditional setting. However, the cryogel takes advantage of this strong binding to only release bound cells while keeping the antibodies attached to the protein A. The application of mechanical stress, recommended using a piston, and compressing the cryogel is reported to detach all cells with antibodies remaining bound to the cryogel-protein A complex for subsequent uses. This helps reduce costs associate with purchasing labeling antibodies. More specifics details on the composition of the cryogel and the operation of the column are described in Section 14.

# **Section 10:**

# **Assembly of Database**



## 10. Assembly of Database

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### 10.1. RBC Conversion Treatment Parameters

The literature that serves as the basis for this project, which describes the methodology and biochemistry of converting any RBC type into the universal type-O, reports that 60 mg of  $\alpha$ -N-acetylgalactosaminidase and 2 mg of  $\alpha$ -galactosidase for every 300 mL of blood are needed to convert type-A and type-B cells, respectively.<sup>29</sup> The average RBC count is  $5 \times 10^6$  RBCs/ $\mu$ L of blood. Although additional glycosidases have been used to accomplish the same conversion, the mass of enzyme required to treat type-A and type-B cells is 30-fold and 1,000-fold less than the next best alternative, respectively.

### 10.2. *E. coli* BL21(DE3) Growth and Protein Yield Parameters

A literature review was performed to determine what protein yield parameters are common in industrial *E. coli* fermentation processes. Emphasis was placed on growth parameters reported in high cell density cultures, which tend to vary from the values presented in cultures with densities of 16 grams of cells per liter. Recent protocols have been published detailing how to obtain high-density *E. coli* cells with yields of 15 to 25 mg of recombinant protein in 50-mL of culture at an OD<sub>600</sub> of 20.<sup>30</sup> (They report that an OD of 1 is approximately  $1.0 \times 10^{10}$  cells/mL.) We assume that our protein yield will be closer to the lower bound. Assuming that we can obtain 15 mg of enzyme per 50-mL of culture, this value corresponds to a protein production rate of approximately 0.30 g protein/L of culture. This corresponds to a yield of  $1.88 \times 10^{-2}$  g of enzyme per gram of cell mass.

Lastly, an additional literature review, both from academic and industrial sources, was performed to determine key operating parameters for modeling the cellular growth of *E. coli* cell cultures. Nearly all literature sources<sup>2</sup> reported *E. coli* growth rates ranging between 0.25 and 0.30 hr<sup>-1</sup>. To have a conservative approach, a growth rate of 0.25 hr<sup>-1</sup> was assumed for this process. The specific oxygen uptake rate for *E. coli* is reported to be 12 mmole O<sub>2</sub>/g cells-hr. These numbers were screened and approved by Jeffrey Cohen, Ph.D., from Johnson & Johnson, for further verification that these values align with common industrial assumptions and yields.

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<sup>29</sup> Liu QP et al. Bacterial glycosidases for the production of universal red blood cells. *Nature Biotechnology* 2007; 25(4): 454-464.

<sup>30</sup> Sivashanmugam A et al. Practical protocols for production of very high yields of recombinant proteins using *Escherichia coli*. *Protein Science* 2009; 18: 936-948.

# **Section 11:**

# **Process Flow Diagrams and**

# **Material Balances**

## 11. Process Flow Diagrams and Material Balances

### 11.1. *E. coli* Upstream and Downstream Processing

Figure 11.1.1 represents the process flow diagram for the upstream processing of the *E. coli* cell lines. Upstream processing refers to the microbial fermentation process in which the *E. coli* cells are grown up to a high enough cell density to express the desired mass of recombinant enzyme. The overall material balances for each reactor are shown in Table 11.1.1. The calculations to determine batch times are found in Appendix A. A summary of all batch culture data for the production of both  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase is included in Appendix D.

**Table 11.1.1.** Overall material balance for each bioreactor or fermenter in the upstream processing.

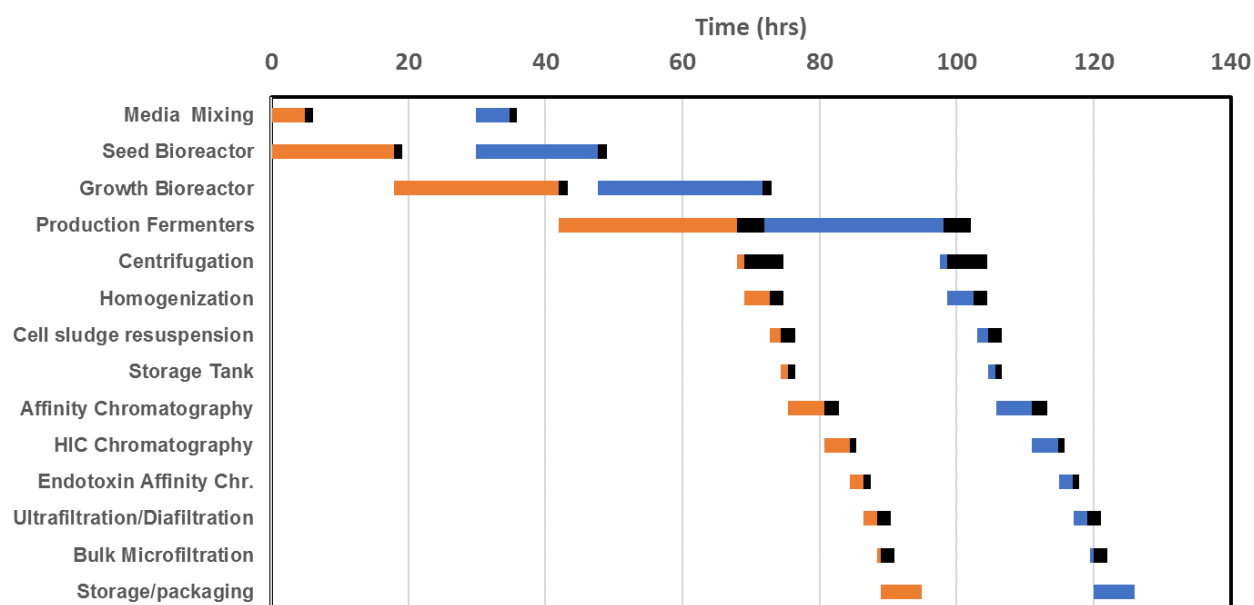
Inoculum Growth		Input (kg/batch)	Output (kg/batch)
	Cells	$5.60 \times 10^{-7}$	$4.87 \times 10^{-5}$
	Nutrients	$9.74 \times 10^{-5}$	0
	Water	0.110	0.105
	Kanamycin	$5.50 \times 10^{-6}$	$5.50 \times 10^{-6}$
	Chloramphenicol	$3.40 \times 10^{-6}$	$3.40 \times 10^{-6}$
	Endotoxin	0	$3.20 \times 10^{-3}$
	Waste	0	$3.39 \times 10^{-4}$
Seed Bioreactor		Input (kg/batch)	Output (kg/batch)
	Cells	$4.62 \times 10^{-5}$	$1.88 \times 10^{-2}$
	Nutrients	$3.76 \times 10^{-2}$	0
	Water	5.44	5.21
	Kanamycin	$2.72 \times 10^{-4}$	$2.72 \times 10^{-4}$
	Chloramphenicol	$1.85 \times 10^{-4}$	$1.85 \times 10^{-4}$
	Endotoxin	$3.20 \times 10^{-3}$	0.158
	Waste	0	0.584
Production Fermenter		Input (kg/batch)	Output (kg/batch)
	Cells	$1.78 \times 10^{-2}$	11.5
	Nutrients	23.1	0
	Water	396	379
	IPTG	$9.44 \times 10^{-2}$	$9.44 \times 10^{-2}$
	GalNAc-ase	0	0.216
	OR $\alpha$ -galactosidase	0	0.199
	Endotoxin	0.158	11.5
	Waste	0	358

Figure 11.1.2 shows the downstream purification process of the ECO-zymes expressed in the *E. coli* cells. The overall material balance of the downstream purification process is shown in Table 11.1.2.

**Table 11.1.2.** Overall material balance for the downstream purification of ECO-zymes.

	Input (kg/batch)	Output (kg/batch)
Cells	11.5	0
Media	379	0
IPTG	$9.44 \times 10^{-2}$	0
Kanamycin	$2.72 \times 10^{-4}$	0
Chloramphenicol	$1.85 \times 10^{-4}$	0
GalNAc-ase	0.205	0.136
OR $\alpha$ -galactosidase	0.189	0.125
Endotoxin	$11.5 \times 10^{-3}$	$1.04 \times 10^{-9}$
Waste	0	391

Figure 11.1.3 shows a Gantt chart showing the time period in which two batches of enzyme production can be completed.



**Figure 11.1.3.** Gantt chart for *E. coli* processing scheduling. Two batches can be achieved in approximately a week. The bottlenecks of the setup are the production fermenters. The color black represents CIP/SIP times. Orange is the first batch, blue is the second batch.

Based on the equipment processing times, it was decided that 112 batches would be done for the production of  $\alpha$ -N-acetylgalactosaminidase and two batches for the production of  $\alpha$ -galactosidase. Additionally, in consultation with several industrial consultants, it was decided that the facility would be operational throughout the entire year, seven days a week. Although the Gantt chart shows that only two batches can be prepared in roughly a week, six days a week is not sufficient to accomplish the entire production goals. There would need to be weeks in which all seven days required labor. For simplicity purposes, it was then decided that the facility should operate seven days a week to meet production goals and to compensate for any delays.

## 11.2. RBC Treatment Processing

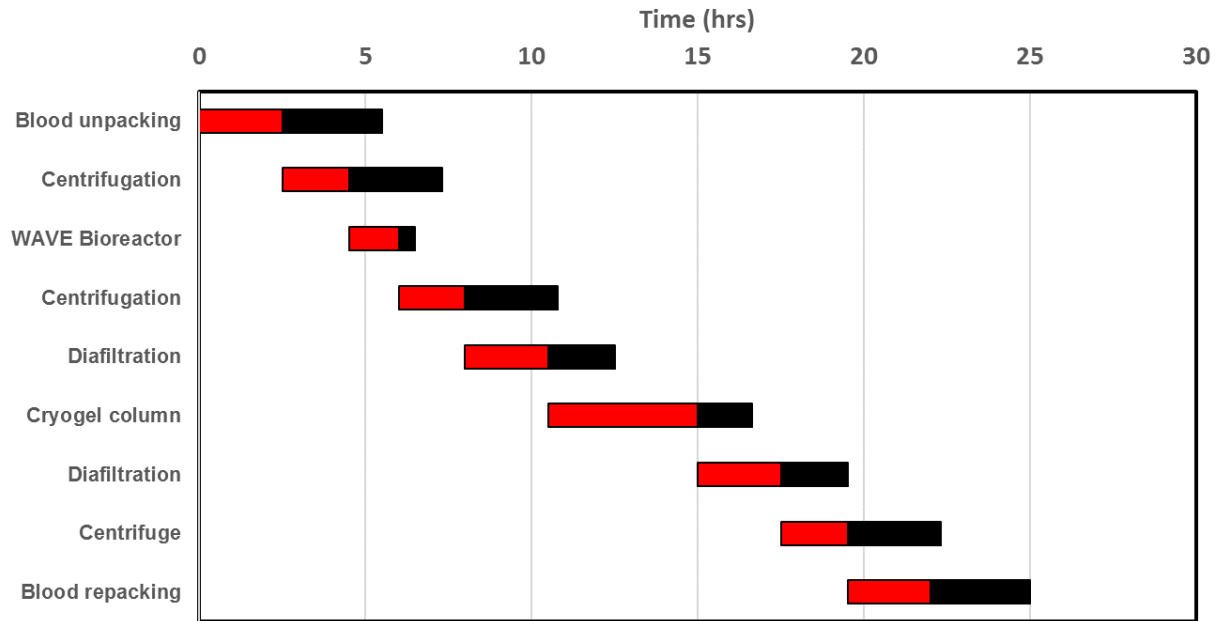
The final process flow diagram in Figure 11.2.1 (at the end of the section) represents the RBC treatment process and its respective downstream purification. The overall material balance is shown in Table 11.2.1.

**Table 11.2.1.** Overall material balance for the key components of the RBC treatment process.

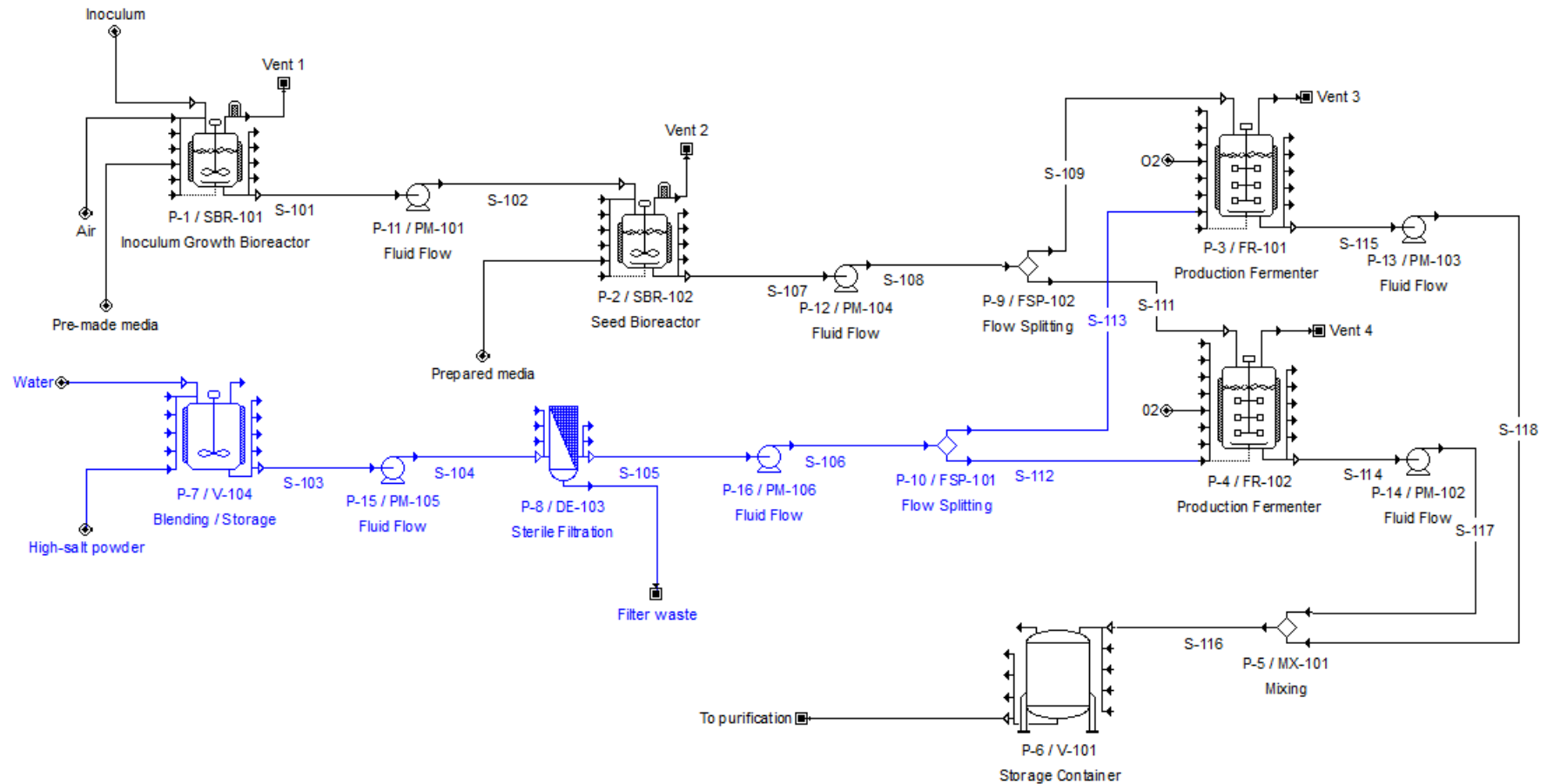
	Input (kg/batch)	Output (kg/batch)
<b>For type-A</b>		
RBC suspension	0.559	0.386
GalNAc-ase	0.118	0
Waste	0	0.291
<b>For type-B</b>		
RBC suspension	0.551	0.381
$\alpha$ -galactosidase	$3.95 \times 10^{-3}$	0
Waste	0	0.174

A Gantt chart for the RBC treatment process is shown in Figure 11.2.2. Only one batch is shown though, given that we are limiting ourselves to only three batches per week. There are therefore very little time constraints to satisfy RBC production goals. Since it was decided that the facility would operate seven days a week to fulfill enzyme production goals, then there will still be sufficient time for three batches of RBC treatment to be carried out since they can be done in parallel to the bacterial growth. Additionally, each batch takes approximately one day, meaning

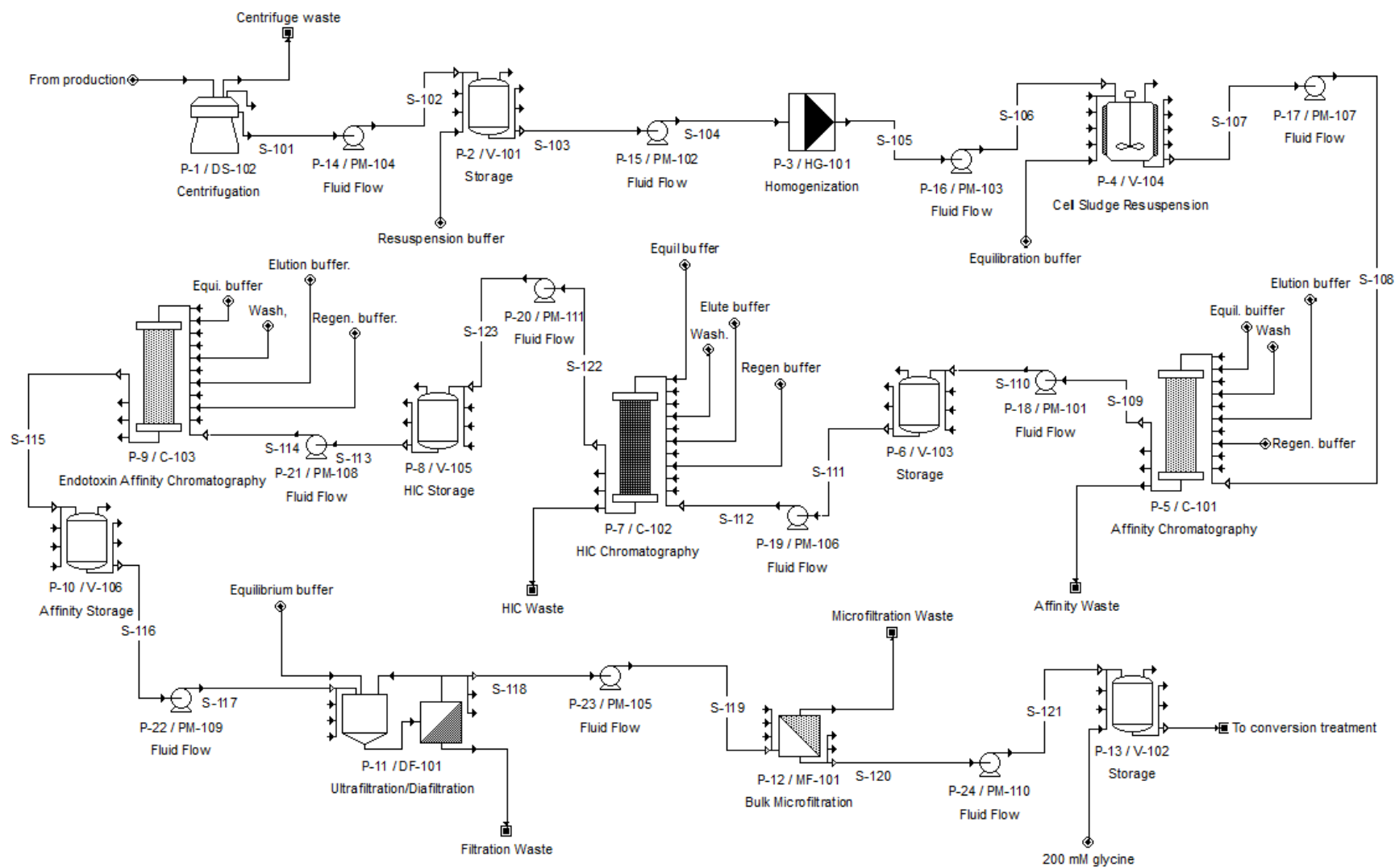
that conversion batches do not need to occur back to back. However, this available free time between each treatment process indicates that there is additional time that can be used later on to increase the number of batches per week if expansion occurs.



**Figure 11.2.2.** Gantt chart for a single batch for RBC conversion. Three batches can easily be achieved in approximately a week, which is what production capacity will limit itself to for the early years of the facility. The color black represents CIP/SIP times, while red represents an RBC conversion process unit operation..

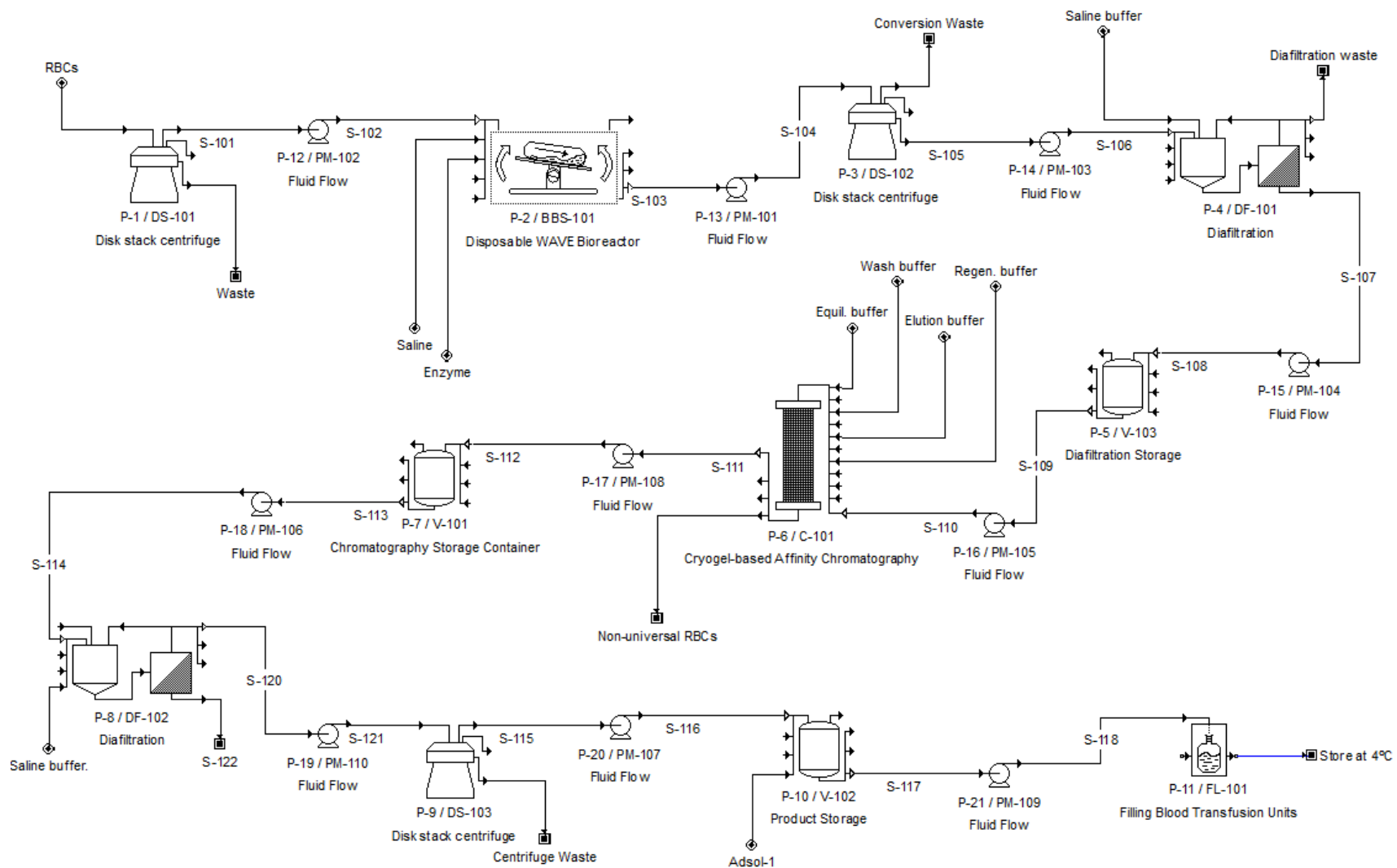


**Figure 11.1.1.** The finalized process flow diagram for upstream cell culture processing of the engineered *E. coli* BL21(DE3) strains expressing either  $\alpha$ -galactosidase or  $\alpha$ -N-acetylgalactosaminidase. Blue represents bulk media preparation and sterilization to charge into fermenters. For the smaller bioreactors, media will be pre-made and stored in sterile conditions.



**Figure 11.1.2.** The finalized process flow diagram downstream purification of either  $\alpha$ -galactosidase or  $\alpha$ -N-acetylgalactosaminidase that is harvested from the *E. coli* BL21(DE3) culture.





**Figure 11.2.1.** The finalized process flow diagram for the RBC treatment conversion process.

# **Section 12:**

# **Process Description**

## 12.A. ECO-enzyme Production Process Description

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The bioreactors and fermenters chosen for this process were designed by finding the production volume required to satisfy production. It is assumed that for all vessels, the culture will only occupy at most 80% of the vessel's volume. It should also be noted that all bioreactors and fermenters purchased in this step come equipped with sampling ports to measure cell density at different sampling times through the growth period. All cell density measurements will be done using a Tecan Spark Microplate Reader, with all measurements being done in replicates to ensure goal cell density has been achieved. It is assumed that any loss of microbial culture due to sampling is negligible.

### 12.A.1. *E. coli* Cell Growth Model for Batch Bioreactor

To analyze cell growth, it was assumed that *Escherichia coli* (*E. coli*) follows Monod growth kinetics, a commonly accepted approach for industrial fermentation. The cell growth times were selected based on a literature review of common experimental values for *E. coli*. The selected growth rate,  $u_g$ , was  $0.25 \text{ hr}^{-1}$ . For simplicity of calculations, it was assumed that the first-order death rate,  $k_d$  is negligible. Therefore, the net growth specific rate was determined as follows:

$$\mu_{net} = u_g - k_d \quad (12.1)$$

The mathematical model used to quantify the batch time for cell growth was:

$$\mu_{net} = \frac{1}{X} \frac{dX}{dt} \quad (12.2)$$

Rearranging the above expression to have the cell concentration and time variables isolated on the left and right-hand sides of the equations, respectively:

$$\frac{dX}{dt} = \mu_{net} X \quad (12.3)$$

Given that the *E. coli* cells are thawed from a frozen stock with a specific concentration, the initial concentration,  $X_0$ , is known. Thus, to solve for the final cell concentration,  $X$ , the integral was taken from  $t = 0$  to  $t$  with their respective cell concentrations.

$$\int_{X_o}^X \frac{dX}{X} = \int_0^t \mu_{net} dt$$

Solving the integral:

$$\ln \frac{X}{X_o} = \mu_{net} t$$

Isolating the batch time required to grow an inoculum of cells at a concentration of  $X_o$  to a final concentration,  $X$ , the resulting equation used was:

$$t = \frac{1}{\mu_{net}} \ln \frac{X}{X_o} \quad (12.4)$$

### 12.A.2. Cell Culture Media Preparation

Literature on high-density microbial cell cultures recommends using high-mineral salt culture media to sustain large cell concentrations.<sup>31</sup> The composition of the culture is included in Appendix Q. All mineral salts will be purchased in dry powder form, dissolved in ultrapure water, and mixed at 300 rpm in a 500 L HyPerforma Single-Use Mixer from Thermo Fisher. The prepared solution will be supplemented with 0.04% vol/vol glycerol and 0.01% vol/vol  $\beta$ -lactose. All samples will then be passed through a 0.2  $\mu$ m filter to sterilize and eliminate any foreign particles, bacteria, or large viruses. All culture media will also be supplemented with 2 grams of glucose per culture of media, as recommended by Jeffrey Cohen, Ph.D (Johnson & Johnson), prior to insertion the microbial bioreactors and fermenters. The total time for this mixing step, including charging all the high-salt powders and water, mixing and heating to 37°C, and sterilization, will take roughly five hours.

For the inoculum grow up and additional grow up in a seed reactor, antibiotics will also be included to eliminate any *E. coli* cells that do not express the genes that code for either  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase. Successfully engineered *E. coli* strains that express the genetic sequence to produce the enzymes of interest also include an attached antibiotic resistance gene. If for any reason any of the *E. coli* cells do not have the genes for enzyme

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<sup>31</sup> Sivashanmugam A et al. Practical protocols for production of very high yields of recombinant proteins using *Escherichia coli*. Protein Science 2009; 18: 936-948.

expression, they will not express the antibiotic resistance gene either. The use of antibiotics will allow only enzyme-producing *E. coli* cells to be selected for the microbial cultures. The working concentrations for kanamycin and chloramphenicol established in the literature are 50 and 34 µg/mL, respectively.<sup>31</sup> It is assumed that after the first culturing steps, the microbial culture will consist exclusively of enzyme-expressing bacterial cells. Therefore, the antibiotics are removed from the production fermentation step to reduce costs.

### 12.A.3. Inoculum Preparation

The inoculum for the growth bioreactor and production fermenter will initially be grown up in a 0.250 L single-use, disposable stirred tank bioreactor operated in batch using the ambr<sup>®</sup> 250 modular system (Sartorius).<sup>32</sup> The process will be carried out with a working volume of 0.11 L inoculated with 1.0 mL of a frozen stock of *E. coli* BL21(DE3) cells at a concentration of  $7.95 \times 10^6$  cells/mL. It is assumed that only 80% of the cells in the frozen vial will survive the freeze-thaw process. After insertion of the inoculum into the culture media and accounting for cell death, the initial concentration of the cell population will be  $5.09 \times 10^6$  cells/mL. The culture will grow for 17.86 hours, regardless of the enzyme product, up until a final cell concentration of  $4.42 \times 10^8$  cells/mL is obtained. The reactor will operate at 300 rpm with a dual 20 mm Rushton impeller and at 37°C achieved via electrical heating. Media in the bioreactor will contain 33.9 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 15.0 g KH<sub>2</sub>PO<sub>4</sub>, 5.0 g NH<sub>4</sub>Cl, 2.5 g NaCl, 7.1 g Na<sub>2</sub>SOL, 6.0 g MgSO<sub>4</sub>, 2 g glucose, 34 mg chloramphenicol, and 50 mg kanamycin per liter of solution. During the inoculation growth, it should be noted the cells will produce small amounts of endotoxin, approximately 3.2 grams. Additionally, roughly 5 milliliters of water in the culture media will be lost due to evaporation.

After the near 18-hour growth period, the inoculum culture will be transferred to a seed bioreactor via peristaltic pump for further grow up prior to insertion into the production fermenters. All the contents of the unit will be transferred into the new growth bioreactor. If for any reason the seed bioreactor is not available for immediate use, the inoculum culture will be transferred to a

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<sup>32</sup> Sartorius Stedim Biotech GmbH. Data sheet: ambr 250 modular bioreactor [Internet]. Data sheet: ambr 250 modular bioreactor. Goettingen, Germany: Sartorius Stedim Biotech GmbH; 2015 [cited 2018 Apr 16]. Available from: [https://www.sartorius.com/\\_ui/images/h0c/h06/8870706315294.pdf](https://www.sartorius.com/_ui/images/h0c/h06/8870706315294.pdf)

single-use disposable bag and refrigerated at 4°C to inhibit further cell growth until inoculation for the growth bioreactor.

#### **12.A.4. Growth Bioreactor**

The growth bioreactor will be inoculated from the *E. coli* culture grown in the single-use bioreactor. The culture will be grown up in an autoclavable glass stirred tank bioreactor operated in batch using a BIOSTAT® B control tower (Sartorius).<sup>33</sup> The process will be carried out with a working volume of 5.44 L in a 10 L UniVessel autoclavable glass vessels<sup>34</sup>, also purchased from Sartorius. Prior to inoculation, 5.33 L of prepared and sterilized high-salt medium will be transferred into the 10 L using a peristaltic pump. Immediately after, the remaining 0.11 L of culture from the inoculum growth step will be charged into the reactor via peristaltic pump. Assuming that approximately 95% of the active microbial population is transferred into the seed bioreactor, after dilution with fresh media the initial cell concentration is  $8.50 \times 10^6$  cells/mL. The remaining five percent that is lost is due to either cell death or transfer losses. Growth will continue for 24.03 hours and 23.69 hours for  $\alpha$ -N-acetylgalactosaminidase and  $\alpha$ -galactosidase, respectively. A maximum cell concentration of  $3.45 \times 10^9$  and  $3.17 \times 10^9$  cells/mL will be obtained in each culture. Media in the seed bioreactor will contain 33.9 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 15.0 g KH<sub>2</sub>PO<sub>4</sub>, 5.0 g NH<sub>4</sub>Cl, 2.5 g NaCl, 7.1 g Na<sub>2</sub>SOL, 6.0 g MgSO<sub>4</sub>, 2 g glucose, 34 mg chloramphenicol, and 50 mg kanamycin per liter of solution. In addition to microbial growth, this setup will also increase the final endotoxin yield thus far to approximately 0.16 grams. It is expected that after the near 24-hour growth period elapses, approximately 23 milliliters of water will have evaporated.

The culture will be stirred continuously at 800 rpm with a 6-blade disk (Rushton turbine) impeller to keep the culture well mixed and allow for an optimal volumetric oxygen transfer, as indicated by the manufacturer's protocol. The final biomass density in the cultures are 3.45 g/L for  $\alpha$ -N-acetylgalactosaminidase and 3.17 g/L for  $\alpha$ -galactosidase, both of which are fairly low density microbial fermentations. The manufacturer's protocol indicates that the BIOSTAT® B

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<sup>33</sup> Sartorius Stedim Biotech GmbH. BIOSTAT® B: The Multi-Talented Bioreactor for Research and Development [Internet]. BIOSTAT® B: The Multi-Talented Bioreactor for Research and Development. Goettingen, Germany: Sartorius Stedim Biotech GmbH; 2014 [cited 2018 Apr 16]. Available from: [https://www.sartorius.com/mediafile/Broch\\_BIOSTAT-B\\_SBI1513-e.pdf](https://www.sartorius.com/mediafile/Broch_BIOSTAT-B_SBI1513-e.pdf)

<sup>34</sup> Sartorius Stedim Biotech GmbH. UniVessel® Glass: Reliability and Continuity [Internet]. UniVessel® Glass: Reliability and Continuity. Goettingen, Germany: Sartorius Stedim Biotech GmbH; 2017 [cited 2018 Apr 16]. Available from: [http://www.emdmillipore.com/Web-US-Site/en\\_CA/-/USD/ShowDocument-File?ProductSKU=MM\\_NF-C173675&DocumentId=201504.204.ProNet&DocumentUID=17731154&DocumentType=DS&Language=EN&Country=NF&Origin=PDP](http://www.emdmillipore.com/Web-US-Site/en_CA/-/USD/ShowDocument-File?ProductSKU=MM_NF-C173675&DocumentId=201504.204.ProNet&DocumentUID=17731154&DocumentType=DS&Language=EN&Country=NF&Origin=PDP)

system is capable of a volumetric oxygen transfer coefficient greater than  $40 \text{ hr}^{-1}$ . Based off the higher cell density, the oxygen transfer rate is  $45.6 \text{ mmole O}_2/\text{g cell-hr}$  while the oxygen uptake rate of the cells under these conditions is  $41.4 \text{ mmole O}_2/\text{g cell-hr}$ . This calculation additionally ensures that the oxygen transfer rates for this bioreactor setting is greater the oxygen uptake rate of the *E. coli* cells, which maximize cell growth and promotes a healthy microbial population for production fermentation. The pH of the culture will be maintained at 7.0; the temperature, at  $37^\circ\text{C}$  with electric heating. Sterile oxygen will be sparged to maintain a dissolved oxygen concentration ( $\text{pO}_2$ ) of 40% throughout the whole growth period. All conditions will be stabilized using PID feedback controllers.

If for any reason the production fermenters are not available for immediate use, the seed bioreactor culture will be transferred to a single-use disposable bag and refrigerated at  $4^\circ\text{C}$  to inhibit further cell growth until inoculation for the growth bioreactor.

#### **12.A.5. Production Fermenter Preparation**

The production fermenters will be inoculated with the *E. coli* culture from the growth bioreactor in the previous step. The culture will be grown in two separate stainless steel BIOSTAT® D-DCU fermenters (Sartorius).<sup>35</sup> First, 391 L of high-salt culture media will be prepared, sterilized, and then transferred into the two stainless steel fermenters in equal volumetric quantities via peristaltic pump. Immediately after the fermenters have been charged with media, the 5.44 L of culture from the growth bioreactor will be separated into two volumetrically equal streams, both of which will be transferred into the fermenters through a peristaltic pump. It is once again assumed that nearly five percent of the microbial population grown in the seed bioreactor will be lost to cell death of transport losses. The culture will be stirred continuously at 800 rpm with a 6-blade disk (Rushton turbine) impeller with an optimal oxygen transfer coefficient of  $308 \text{ hr}^{-1}$ , allowing a maximum cell biomass density of 29 grams per liter to be supported (see Appendix F). These operating conditions were chosen following the manufacturer's protocol and published data, which report optimal *E. coli* BL21(DE3) cell growth and viability when operating at high impeller velocities.

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<sup>35</sup> Sartorius Stedim Biotech GmbH. BIOSTAT® D-DCU: Your "Fast Lane" to Production [Internet]. BIOSTAT® D-DCU: Your "Fast Lane" to Production. Goettingen, Germany: Sartorius Stedim Biotech GmbH; 2015 [cited 2018 Apr 16]. Available from: [https://www.sartorius.com/mediafile/Broch\\_Biostat\\_D-DCU\\_SBI1512-e.pdf](https://www.sartorius.com/mediafile/Broch_Biostat_D-DCU_SBI1512-e.pdf)

The pH of the culture will be maintained at 7.0; the temperature, at 37°C with the fermenters' integrated heat jackets. Sterile oxygen will be sparged to maintain a dissolved oxygen concentration (pO<sub>2</sub>) of 40% throughout the whole growth period. All conditions will be stabilized using PID feedback controllers.<sup>36</sup> The culture will be grown for 25.89 hours to reach a final cell concentration of  $2.91 \times 10^{10}$  cells/mL. This corresponds to a cell mass of approximately 11.5 kg. Media in the all bioreactors will contain 33.9 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 15.0 g KH<sub>2</sub>PO<sub>4</sub>, 5.0 g NH<sub>4</sub>Cl, 2.5 g NaCl, 7.1 g Na<sub>2</sub>SOL, 6.0 g MgSO<sub>4</sub>, and 2 g glucose per liter of culture media broth. Note that in this unit operation, the kanamycin and chloramphenicol antibiotics are no longer included. After the seed growth, it is assumed that any cells that do not contain the plasmid coding for the gene of interest and antibiotic resistance are eliminated. If for any reason any of these cells do remain, they are found in a exorbitantly small proportion compared to the engineered *E. coli* strain, meaning that their growth in culture will be minimal. The exclusion of these expensive antibiotics in the product fermentation step also reduces costs.

When the desired optical density measurements (OD<sub>600</sub>) value is obtained, isopropyl β-D-1-thiogalactopyranoside (IPTG) will be added to the culture to induce protein production. IPTG will be transferred in the fermenters until a final concentration of 1.0 mM for the working volume is achieved. Protein production will occur for five hours with the culture conditions at a temperature of 25°C, pH of 7.0, and a pO<sub>2</sub> of 40%. The culture will continue to be mixed at 800 rpm. Assuming that the cell concentration remains approximately constant throughout the protein production period, with a cell productivity of 85.4 ng of α-N-acetylgalactosaminidase per cell per day, 216 grams of α-N-acetylgalactosaminidase enzyme can be produced. Under the same assumption with a cell productivity of 85.6 ng of α-galactosidase per cell per day, one batch can produce 199 grams of α-galactosidase. In addition to enzyme production, the microbial fermentation produces 11.5 grams of lipopolysaccharide endotoxins.

After the five hour protein production period, the full cell culture will be transferred via peristaltic pump to a 500 L HyPerforma Single-Use Mixer and cooled to 4°C via the mixer's heated jacket. However, given the transport losses when passing the microbial culture to downstream purification and processing, another five percent of the microbial population will be lost. Therefore,

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<sup>36</sup>Temperature/PID Controllers [Internet]. Omega. Omega; 2018 [cited 2018Apr17]. Available from: <https://www.omega.com/guides/tempcontrol.html>



the mass of enzyme available to harvest once the microbial culture is charged into the holding tank is 205 grams of N-acetylgalactosaminidase or 189 grams of  $\alpha$ -galactosidase. Once the fermenters' contents are transferred out completely, CIP/SIP procedures will begin immediately afterwards.

## 12.B. ECO-zyme Purification Process Description

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It should be noted that for all chromatography column designs, a conservative assumption made regarding the mass of enzyme that the column will treat. The amount of enzyme lost in the previous downstream steps is neglected, leaving us to calculate required resin and column volumes using the enzyme mass that originally entered the downstream process. This is done to calculate the maximum volume of resin and column that would be required to accomplish the desired separation. This serves as the upper bound for costing calculations, which will be usefully in providing more constraints to gauge the financial feasibility of the project. Additionally, this approach will help ensure that there is a surplus of resin if ever required.

Another important note for this section is that the values reported for resin and chromatography column volumes correspond to the calculations done for  $\alpha$ -N-acetylgalactosaminidase purification. Since a single batch of  $\alpha$ -N-acetylgalactosaminidase has a higher mass yield than a batch of  $\alpha$ -galactosidase (205 grams versus 189 grams), it can be expected that all designs parameters for  $\alpha$ -N-acetylgalactosaminidase purification will be greater than the minimum volume needed for  $\alpha$ -galactosidase purification. Therefore a single chromatography column can be used for the purification of both enzymes.

The last major calculation done in this section assumes that the hydrophobic interaction chromatography column used to eliminate endotoxins from the enzyme product will process the original endotoxin mass yield from the microbial fermentation. Similarly to how the resin volumes were calculated, this assumption provides the upper bound on how much resin will be required for the separation step. Additionally, this assumption allows for a more stringent purification process to make sure that the process design's enzymatic product complies with FDA endotoxin standards.

### 12.B.1. Holding Vessel

Upon completion of upstream enzyme production, the entire spent media and culture with the product from both stainless steel fermenters (396 L for  $\alpha$ -N-acetylgalactosaminidase or 364 L for  $\alpha$ -galactosidase) will be transferred via peristaltic pump to a 500 L HyPerforma Single-Use Mixer. This unit was chosen for its single-use technology, which consists of harvesting cells in single-use disposable bags to reduce CIP times, and its heated jacket for temperature control. The culture will be mixed at 300 rpm on a WAVE bioreactor rocker table to prevent settling of the cells

and to minimize loss during transfer out of the holding tank via peristaltic pump to centrifugation. The culture will also be lowered to 5°C, the lower limit of the operating temperature for the vessel, to completely inhibit further cell growth. The vessel will also operate at a pressure of 0.03 atm.<sup>37</sup>

### **12.B.2. Centrifugation**

The content held in the holding tank will be transferred into a Culturefuge100 disk stack centrifuge, purchased from Alfa Laval. The unit will separate the solid parts of the culture, including cell mass and debris, from the spent liquid media. The spent culture media will then be directed into a waste tank, while the solid biomass will remain in a sludge that is 60% by mass water and 40% by mass solids.<sup>38</sup> The cell biomass will be directed to a disposable 3D bioprocess container (Thermo Fisher Scientific)<sup>39</sup> and kept at 4°C until it undergoes homogenization via microfluidization. The centrifuge will operate with a flowrate of 100 L/hr at a temperature of 25°C. It is assumed that 5% of the ECO-zyme will be lost in the liquid waste stream, leading to a product yield of 95%. Centrifugation will last for approximately one hour, while CIP and SIP procedures will require 5.8 hours as recommended by the manufacturer's protocol.

It is assumed that approximately 4.5% of the biomass will be lost from the centrifugation process. Thus, after centrifugation, roughly 10.9 kg of biomass will be available for harvesting, meaning that the mass of enzyme that can be harvested lowers to 195 grams of  $\alpha$ -N-acetylgalactosaminidase or 180 grams of  $\alpha$ -galactosidase. It is also assumed that the same amount of endotoxin is lost, leaving 10.9 grams of endotoxin. After the centrifugation process, the expected mass of the cell sludge output is approximately 12.7 kg.

### **12.B.3. High-Pressure Homogenization**

The cell sludge from the centrifugation step will be sent through a high-pressure microfluidizer processor (Microfluidics)<sup>40</sup> to lyse the *E. coli* cells and release the internalized,

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<sup>37</sup> Thermo Scientific. Data Sheet: HyPerforma 500 L Single-Use Mixer [Internet]. Data Sheet: HyPerforma 500 L Single-Use Mixer. Waltham, MA: Thermo Scientific; 2017 [cited 2018 Apr 16]. Available from: <https://www.thermofisher.com/order/catalog/product/SUM0500.9002>

<sup>38</sup> Alfa Laval AB. Culturefuge 100 Solid Ejecting Centrifuge [Internet]. Culturefuge 100 Solid Ejecting Centrifuge. Lund, Sweden: Alfa Laval AB; 2018 [cited 2018 Apr 16]. Available from: <https://www.alfalaval.com/globalassets/documents/products/separation/centrifugal-separators/disc-stack-separators/culturefuge-100.pdf>

<sup>39</sup> Thermo Scientific. Single-Use BioProcess Containers [Internet]. Single-Use BioProcess Containers. Waltham, MA: Thermo Scientific; 2017 [cited 2018 Apr 16]. Available from: <https://www.thermofisher.com/search/results?query=3D+bioprocess+containers&sort=relevancy&persona=DocSupport&focusarea=Search+All>

<sup>40</sup> Microfluidics. M-7125, M-7250 Enhanced BioPharmaceutical Microfluidizer® Processor [Internet]. M-7125, M-7250 Enhanced BioPharmaceutical Microfluidizer® Processor. Westwood, MA: Microfluidics International Corporation; 2010 [cited 2018Apr16]. Available from: <https://www.microfluidicscorp.com/sites/default/files/700bp-enhanced.pdf>

cytoplasmic  $\alpha$ -galactosidase or  $\alpha$ -N-acetylgalactosaminidase. 326 L of bacterial protein extraction agent (B-PER) and DNase solution will be added to the disposable bioprocess container in which the cell sludge from centrifugation is stored. (It is assumed that the BPER and DNase solution has the same density as water.) This membrane disrupting solution is added prior to entering the microfluidizer to further weaken the cell membrane and promote cell lysis. The microfluidizer will pump the cell sludge through a pressure differential of up to 20,000 psi in fixed-geometry microchannels to generate high velocity and shear stress. The impact against the microchannels' with the high shear conditions will cause cells membranes to break, releasing the internalized protein. The microfluidizer will operate at 25°C to prevent denaturing of the enzyme. The temperature will be controlled and maintained using a digital PID controller. This process will require three passes to create a uniform emulsion. The total homogenization process, including SIP and CIP procedures, will require approximately 3.8 hours. CIP/SIP procedures are recommended to last for two hours. It is once again assumed that the recovery of the product in the unit operation is roughly 95%.

#### **12.B.4. Microfluidization Product Resuspension Tank and Container**

The lysed cell mass and released enzyme product will be emptied into a new disposable 500 L 3D bioprocess container fitted into a 500 L HyPerforma Single-Use Mixer (Thermo Fisher Scientific). The biomass suspension will be resuspended in 396 L of equilibration buffer to maintain its pH at 7.4. It is once more assumed that product recovery in this step is 95.5%, meaning that 185 grams of  $\alpha$ -N-acetylgalactosaminidase or 171 grams of  $\alpha$ -galactosidase will be collected. To prevent the lysed biomass and product enzymes from settling, which could result in lower product yield, the production suspension is gently agitated at 300 rpm on a WAVE bioreactor rocker table. This agitation speed is reduced enough to not generate shear stress that could otherwise compromise the biological functionality of the enzyme proteins. The operation time, including CIP and SIP sterilization, will be approximately one hour. If the cobalt affinity chromatography is not immediately available due to unexpected bottleneck constraints, mechanical malfunctions, or other issues, the holding bag and its content will be kept in a cold room at 4°C.

### 12.B.5. Cobalt Affinity Chromatography

As previously mentioned, the gene sequence encoded into the *E. coli* BL21(DE3) cell creates an N-terminal truncated construct of the enzymes with a His<sub>6</sub> tag at the N-terminus that can be used for separation procedures. After complete cell lysis and resuspension of both cell debris and product in equilibration buffer, cobalt ion affinity chromatography will be performed to separate  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase from the cell debris, BPER-DNAse mixture, and other buffers. The His<sub>6</sub> tags at the N-terminus of the enzymes will bind to the resin in the chromatography column, while all non- His<sub>6</sub> tagged particles, debris, and organelles will pass through the column.

As previously mentioned, for the purpose of designing the chromatography, we assume that the mass of enzyme that will be processed is equal to the mass of the enzyme at the beginning of the downstream purification. This solves for the upper bound of resin and column volume that would be needed for purification and separations. The total required chromatography column size to treat one batch, which yields 205 grams of enzyme, is 14.6 L. Resolute<sup>®</sup> chromatography columns will be used for this unit procedure and will be purchased from Pall Corporation. The column will be packed with HisPur Cobalt Resin<sup>41</sup>, a tetradentate chelating agarose resin charged with divalent cobalt (Co<sup>2+</sup>) for obtaining high-purity His<sub>6</sub>-tagged proteins, from Thermo Fisher Scientific. The volume of resin required per batch is 10.25 L. Assuming product recovery of 95.5%, the mass collected is 176 grams of  $\alpha$ -N-acetylgalactosaminidase or 162 grams of  $\alpha$ -galactosidase. It is assumed that all cell debris and antibiotic mass is eliminated in this unit operation. Lastly, the affinity chromatography column will operate a linear velocity of 150 cm/hr and at a temperature of 25°C. The chromatography separation, including resin set up, will require 5.27 hours. CIP and regeneration will take an additional 3.14 hours.

For operating, the column must first be run with ten column volumes of equilibration buffer. The resuspended solution of cell debris and product in equilibration buffer will then be added to the column to allow for protein binding to the resin. The resin has an expected binding capacity of 20 grams of enzyme per liter of resin for both  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase. After binding, the column will then be washed with ten volumes of wash

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<sup>41</sup> Pall Life Sciences. Resolute<sup>®</sup> Manual Chromatography Columns [Internet]. Resolute<sup>®</sup> Manual Chromatography Columns. Port Washington, NY: Pall Corporation; 2017 [cited 2018 Apr 16]. Available from: <https://shop.pall.com/us/en/biotech/chromatography/columns-pilot-and-process-scale/resolute-manual-chromatography-columns-zidgri78lcv>

buffer to eliminate any cell debris that may have bound to the resin. Lastly, ten column volumes of elution buffer will be added to the column to release and collect all bound enzyme.

Regeneration of the resin will be done by washing the column and resin with ten column volumes of regeneration buffer. According to the manufacturer's protocol, the resin can be regenerated a maximum of 25 times without decreasing the purity of the product below 90%.

#### **12.B.6. Hydrophobic Interaction Chromatography**

The removal of endotoxins, the pyrogenic components of the outer cell wall of Gram-negative bacteria such as *E. coli*, is necessary to prevent inducing immunogenic responses in patients. Since  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase will be used to treat RBCs and could possibly remain in solution with them after the conversion, transfusion of these units could theoretically cause significant levels of endotoxin contamination. Although endotoxin removal is reported when cell solutions are run through a cobalt affinity chromatography column, this process operates under the assumption that the endotoxin removal is negligible. This assumption is made given that the cobalt-chelate coordination core is reported to have off-target binding to endotoxins and other protein host contaminants.

This column will be packed with POROS Benzyl HIC Resin<sup>42</sup>, which consists of cross-linked poly(styrene-divinylbenzene) POROS base beads with aromatic hydrophobic benzyl ligand, suited for both bind/elute and flow-through purifications. The benzyl resin system was chosen over other available resins because binding affinity can easily be controlled even with relatively small differences in hydrophobicity. Assuming that no endotoxin has been removed since the beginning of the downstream purification to be stricter on design calculations, each batch of *E. coli* BL21(DE3) culture produces 11.5 grams of endotoxin to be processed. Therefore, the volume of resin required for each batch HIC chromatography operation is 57.5 L. The total required chromatography column size to treat one batch is 96 L. Resolute<sup>®</sup> chromatography columns will also be used for this unit procedure. Lastly, the endotoxin affinity chromatography column will also operate a linear velocity of 150 cm/hr and at a temperature of 25°C. The chromatography separation, including resin set up, will require 3.79 hours. CIP and regeneration will take

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<sup>42</sup> Thermo Scientific. Instructions: HisPur™ Cobalt Resin [Internet]. Instructions: HisPur™ Cobalt Resin. Rockford, IL: Pierce Biotechnology; 2012 [cited 2018 Apr 16]. Available from: <https://www.thermofisher.com/order/catalog/product/89964>

approximately one hour. Although the manufacturer's protocol for this resin reports a 99% removal of endotoxins, other sources report that only a 90% removal was often obtained. For this reason, it is assumed that the hydrophobic interaction column will only remove 90% of the endotoxins from the enzyme product. After processing, 1.15 grams of endotoxin will remain. Assuming approximately 95% enzyme product recovery, either 167 grams of  $\alpha$ -N-acetylgalactosaminidase or 162 grams of  $\alpha$ -galactosidase will be collected.

For operating, the column must first be run with five column volumes of equilibration buffer. The collected product solution from the previous chromatography step will then be added to the column to allow for endotoxin binding to the resin. After binding, the column will then be washed with five volumes of wash buffer to eliminate any cell debris that may have bound to the resin. Lastly, two column volumes of elution buffer will be added to the column to release and collect all bound enzyme. CIP procedures include adding two column volumes of 6M guanidine HCl<sup>43</sup> and another two column volumes of 1% mass/volume Brij35 nonionic detergent.<sup>44</sup>

Regeneration of the resin will be done by washing the column and resin with five column volumes of regeneration buffer. According to the manufacturer's protocol, the resin can be regenerated a maximum of ten times without any loss in performance.

#### **12.B.7. Endoxotin Affinity Chromatography**

The hydrophobic interaction chromatography (HIC) unit operation alone by itself cannot sufficiently reduce the endotoxin concentration in the product suspension to the FDA standard of 0.1 EU/mL. Therefore, an endotoxin affinity chromatography operation was added to the process to further remove endotoxins from the product.

The last column will be packed with Pierce High Capacity Endotoxin Removal Resin (Thermo Fisher Scientific)<sup>45</sup>, which combines porous cellulose beads with poly( $\epsilon$ -lysine) as an affinity ligand to selectively bind endotoxins. The binding affinity is also reported to be  $2 \times 10^6$  EU/mL, where EU is shorthand for endotoxin units. After the HIC separation step, 1.15 grams of

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<sup>43</sup> Thermo Scientific. User Guide: POROS™ HIC Resins: Ethyl, Benzyl, and Benzyl Ultra [Internet]. User Guide: POROS™ HIC Resins: Ethyl, Benzyl, and Benzyl Ultra. Waltham, MA: Thermo Scientific; 2017 [cited 2018 Apr 16]. Available from: <https://www.thermofisher.com/order/catalog/product/88272?SID=srch-srp-88272>

<sup>44</sup> Brij 35 for Synthesis; MSDS No. 801962 [Online]; EMD Millipore: Saint Louis, MO, Sep 16, 2014. [http://www.emdmillipore.com/Web-US-Site/en\\_CA/-/USD/ProcessMSDS-Start?PlainSKU=MDA\\_CHEM-801962&Origin=PDP](http://www.emdmillipore.com/Web-US-Site/en_CA/-/USD/ProcessMSDS-Start?PlainSKU=MDA_CHEM-801962&Origin=PDP) (accessed Apr 10, 2018).

<sup>45</sup> Thermo Scientific. Instructions: Pierce High-Capacity Endotoxin Removal Resin [Internet]. Instructions: Pierce High-Capacity Endotoxin Removal Resin. Rockford, IL: Pierce Biotechnology; 2016 [cited 2018 Apr 16]. Available from: <https://www.thermofisher.com/order/catalog/product/88272?SID=srch-srp-88272>

endotoxin will need to be treated. Therefore, the volume of resin required per batch is 5.75 L. The total required chromatography column size to treat one batch is 9.6 L. Resolute® chromatography columns will be used for this unit procedure and will be purchased from Pall Corporation. Lastly, the endotoxin affinity chromatography column will also operate a linear velocity of 150 cm/hr and at a temperature of 25°C. The chromatography separation, including set up, will require 3.01 hours, while CIP and regeneration will last slightly over an hour. It is assumed that there is a 99% removal of endotoxins in this unit operation, as indicated in the manufacturer's protocol. After passing the enzyme product solution from the previous unit, either 159 grams of  $\alpha$ -N-acetylgalactosaminidase or 146 grams of  $\alpha$ -galactosidase will be collected.

For operating, the column must first be run with five column volumes of equilibration buffer. The collected product solution from the previous chromatography step will then be added to the column to allow for endotoxin binding to the resin. After binding, the column will then be washed with five volumes of wash buffer to eliminate any cell debris that may have bound to the resin. Lastly, two column volumes of elution buffer will be added to the column to release and collect all bound enzyme. CIP procedures include adding two column volumes of 6M guanidine HCl and another two column volumes of 1% mass/volume Brij35 nonionic detergent.

Regeneration of the resin will be done by washing the column and resin with two column volumes of regeneration buffer. According to the manufacturer's protocol, the resin can be regenerated a maximum of five times without any loss in performance.

#### **12.B.8. Ultrafiltration/Diafiltration**

After separating  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase from endotoxins via several chromatography steps, the next major unit operation is separating these enzymes from any remaining cellular debris and the buffer components from the endotoxin affinity chromatography step. An ultrafiltration/diafiltration setup will be prepared using a Cogent Process-Scale Tangential Flow Filtration System (EMD Millipore)<sup>46</sup> loaded with Pellicon 3 Cassettes with Ultracel Membrane.<sup>47</sup> The membrane will have a 30 kD nominal molecular weight limit with C-screen. The

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<sup>46</sup> EMD Millipore. Data Sheet: Cogent® Process-Scale Tangential Flow Filtration System [Internet]. Data Sheet: Cogent® Process-Scale Tangential Flow Filtration System. Darmstadt, Germany: EMD Millipore; 2015 [cited 2018 Apr 16]. Available from: [http://www.emdmillipore.com/US/en/product/Cogent-Process-Scale-Tangential-Flow-Filtration-System,MM\\_NF-C173675#documentation](http://www.emdmillipore.com/US/en/product/Cogent-Process-Scale-Tangential-Flow-Filtration-System,MM_NF-C173675#documentation)

<sup>47</sup> Millipore Sigma. Pellicon® 3 Cassettes with Ultracel® Membrane [Internet]. Pellicon® 3 Cassettes with Ultracel® Membrane . Burlington, MA: Millipore Sigma; 2018 [cited 2018 Apr 16]. Available from: [http://www.emdmillipore.com/US/en/product/Pellicon-3-Cassettes,MM\\_NF-C9947?CatalogCategoryID=#documentation](http://www.emdmillipore.com/US/en/product/Pellicon-3-Cassettes,MM_NF-C9947?CatalogCategoryID=#documentation)



Ultracel membranes have a surface area of 1.14 m<sup>2</sup> and can be run with a flow rate of 4 L/min. As the process is carried out, the feed tank will be continuously replenished with equilibration buffer. It is assumed that roughly 95% percent of the product will be recovered, while the remaining five percent will be lost to the permeate. This means that either 151 grams of  $\alpha$ -N-acetylgalactosaminidase or 139 grams of  $\alpha$ -galactosidase will be collected. The ultrafiltration/diafiltration step will require approximately two hours. The operation time of the ultrafiltration/diafiltration unit will be roughly two hours, with an equivalent amount of time used for CIP/SIP procedures.

### **12.B.9. Sterile, Bulk Microfiltration**

Sterile filtration is used as the final purification step to remove endotoxins and any remaining microscopic cell debris and organelles from the final product solution. This step is taken to ensure that the final concentration of endotoxins in the ECO-zyme solution meets FDA standards. The filters will be hydrophobic Durapore PVDF membranes with a 0.22  $\mu$ m pore diameter size.<sup>48</sup> The PVDF filters and their respective holders are disposable, and one of each will be used per batch. The filters have a 100 cm<sup>2</sup> surface area and will run at a flowrate of 11 L/min. Recovery of ECO-zyme product in this step is 95%. This unit procedure will be run twice as additional precaution to ensure that FDA requirements for endotoxin removal are satisfied. It is assumed that 99% of endotoxins are removed after each filtration pass. After this unit operation,  $1.04 \times 10^{-6}$  grams of endotoxin will remain. 136 grams of  $\alpha$ -N-acetylgalactosaminidase or 125 grams of  $\alpha$ -galactosidase will be collected. The final concentration of the enzyme product solution after passing through bulk microfiltration will be 1 mg/mL and will be stored in 200 mM glycine solution to prevent the protein from aggregating in solution. Several filters will be run in parallel to allow this unit operation to last approximately 30 minutes. All filters are disposable, but preparation of new units is estimated to last approximately two hours.

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<sup>48</sup> EMD Millipore. Data Sheet: Millidisk® Cartridges and Millipak® Disposable Capsules [Internet]. Data Sheet: Millidisk® Cartridges and Millipak® Disposable Capsules. Westwood, MA: EMD Millipore; 2015 [cited 2018 Apr 16]. Available from: [http://www.emdmillipore.com/US/en/product/Durapore-Membrane-Filters,MM\\_NF-C7631?CatalogCategoryID=#documentation](http://www.emdmillipore.com/US/en/product/Durapore-Membrane-Filters,MM_NF-C7631?CatalogCategoryID=#documentation)

## 12.C. Red Blood Cell Conversion Process Description

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To enzymatically convert type-A and type-B RBCs,  $\alpha$ -N-acetylgalactosaminidase and  $\alpha$ -galactosidase, respectively, will be added to pooled transfusion units. The RBC units will be acquired in variable sized transfusion bags from suppliers such as hospitals, regional blood banks, or the Red Cross, with an average size of 300 mL per transfusion unit. The production goal is 200,000 enzymatically converted RBC transfusion units. Across the entire treatment process, however, there is an assumed 35% loss of RBCs due to lysis and transfer losses. Thus, additional transfusion units will be required to account for the losses to be able to satisfy production goals. Therefore, the process system is designed to operate with an annual working volume associated with approximately 308,000 transfusion units.

One important aspect of this process is that all steps, with the exception of the actual conversion step, are done at 4°C to preserve the biological activity of the RBCs. This decision was made following the most stringent medical standards for transfusion medicine, which are found in the United Kingdom. There, transfusion units that have been outside of a controlled temperature of 4°C for longer than four hours are to be eliminated. This is done in precaution of adversely altering the biological activity and functionality of the RBCs.

### 12.C.1. Hyperbaric Chamber for Blood Bag Emptying

The first step in the RBC conversion process is the collection of the RBC units for future treatment steps. Each batch of enzymatically converted RBCs uses 1,973 units, all of which need to be pooled in a storage vessel prior to downstream treatment. Currently available methods for emptying RBC units, however, are only meant for single bags. These include gravity aided emptying, blood pressure cuffs, pressure infusers, or manual emptying. Given the scale of this process, however, there is a high risk of human error and a requirement for a significant workforce to accomplish this goal.

The proposed solution to efficiently empty many RBC units in parallel uses the same mechanism as commercially available pressure infusers, which empty blood bags using a pressure differential. They operate by surrounding a single RBC unit and increasing the pressure by 300 mmHg, achieve flow rates of  $257 \pm 54$  mL/min. Using this technology as a base, a hyperbaric chamber will be developed to generate a pressure differential of 1.0 atm and generate pressure-

driven flow from the blood bags to the storage vessel. RBC units will be stored on carts that will carry a total of 1,973 units per batch and will be rolled into the hyperbaric chamber. This is approximately the same regardless of which blood type that is being treated. The chamber will be similar to commercially available units but will be designed to contain a storage cart, an exit for the tube bundle to which the blood bags will be connected, and reach an operating pressure of 1.5 atm and temperature of 4°C. Eight rounds of pressurization will need to be performed per batch. Each run will take approximately ten minutes. The entire RBC unit emptying step will take roughly 2.5 hours. Three hours will be allotted to proper CIP/SIP procedures to eliminate any bloodborne biological waste that may remain in the system. This time also includes disposal of the original blood bags. The chamber will be designed and constructed by a third party vendor, with an initial estimated cost of \$100,000 after consultation with numerous industrial consultants. 8.00% of sales will also be allocated to research expenses, which will place a heavy emphasis on the optimization of this process to enhance the speed with which the blood units are emptied.

#### **12.C.2. Centrifugation**

The contents of the RBC storage vessel will be transferred into a Culturefuge 100 disk stack centrifuge via a Quattroflow quaternary diaphragm pump. The centrifuge will separate the solid RBC components from the additive solution waste in which they were suspended. The spent additive solution will be directed to a waste tank for safe disposal. The RBC pellet will be transferred to a new single-use, disposable holding bag and resuspended to its original volume in a 0.9% NaCl solution with 140 mL of CPDA-1 anticoagulant for every liter of blood treated. The stored contents will be kept at 4°C until the next major unit step. The centrifuge will operate with a minimal flowrate of 100L/hr to minimize RBC lysis at a temperature of 4°C. It is assumed that 5% of the RBCs that pass through this unit operation will lyse, leading to a product yield of 95%. Centrifugation will last for two hours to handle the entire blood volume but at lower velocities to prevent shearing. CIP/SIP procedures will require 2.8 hours.

#### **12.C.3. Conversion WAVE Bioreactors**

The RBCs from the disposable holding bag will be transferred into six 100 L WAVE bioreactors via a Quattroflow quaternary diaphragm pump.<sup>49</sup> The initial temperature of the reactor

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<sup>49</sup> Holland Applied Technologies. Spec Sheet QF1200S-CV [Internet]. Spec Sheet QF1200S-CV. Chicago, IL: Holland Applied Technologies; 2015 [cited 2018 Apr 16]. Available from: [http://www.hollandapt.com/Documents/Ctrl\\_Hyperlink/doccopy11285\\_uid192018402382.pdf](http://www.hollandapt.com/Documents/Ctrl_Hyperlink/doccopy11285_uid192018402382.pdf)

will be set at 4°C. As soon as the RBC contents have been emptied into the WAVE bioreactors, the temperature will be programmed to increase in increments of 0.5°C every 45 seconds until a final temperature of 25°C has been reached. This temperature increase interval is the common standard employed in blood warmers to prevent RBCs from lysing due to drastic temperature changes. When the temperature of the contents in the WAVE bioreactors has reached 25°C, pre-warmed  $\alpha$ -N-acetylgalactosaminidase for type-A RBCs, or  $\alpha$ -galactosidase for type-B cells, will be added to the WAVE bioreactors. 60 mg of  $\alpha$ -N-acetylgalactosaminidase will be required for every 0.3 L of type-A RBC solution treated (the equivalent of one transfusion unit), while only 2 mg of  $\alpha$ -galactosidase will be needed to treat the same volume of type-B RBC solution. The conversion reaction will take place for one hour. The WAVE bioreactors will operate at 25 rpm at an angle of 8° to minimize foaming. The pH will be maintained between 7.4 and 7.6. Once the reaction has been completed, the temperature of the WAVE bioreactor and its content will be decreased to 4°C in decreasing intervals of 0.5°C every 45 seconds. For scheduling purposes, an additional 30 minutes to the hour reaction time is allotted for charging all red blood cells into the devices.

#### **12.C.4. Centrifugation**

One hour after the enzymatic conversion has elapsed and as soon as the treated RBCs have been cooled to 4°C, the contents of the WAVE bioreactor will be transferred to a Culturefuge 100 disk stack centrifuge via a Quattroflow quaternary diaphragm pump to separate the treated RBCs from the enzyme in solution. The centrifuge will operate at 100 L/hr and at 4°C. The spent anticoagulant-saline solution with the conversion enzyme will be charged to a waste tank for safe disposal. The remaining solid RBC mass, expected to be 60% by mass saline-anticoagulant solution and 40% by mass solids, will be transferred into a single-use, disposable HyPerforma Smartainer 3D Bioprocess Container (BPC) and resuspended to its original volume again with in a 0.9% NaCl solution with 140 mL of CPDA-1 anticoagulant for every liter of blood treated. Centrifugation will last for two hours to handle the entire blood volume but at lower velocities to prevent shearing. CIP/SIP procedures will require 2.8 hours.

#### **12.C.5. Diafiltration**

To remove any remaining enzyme from the resuspended RBCs, diafiltration will be used. Pellicon 2 Durapore PVDF microfiltration filters will be used with a 500 kD nominal molecular

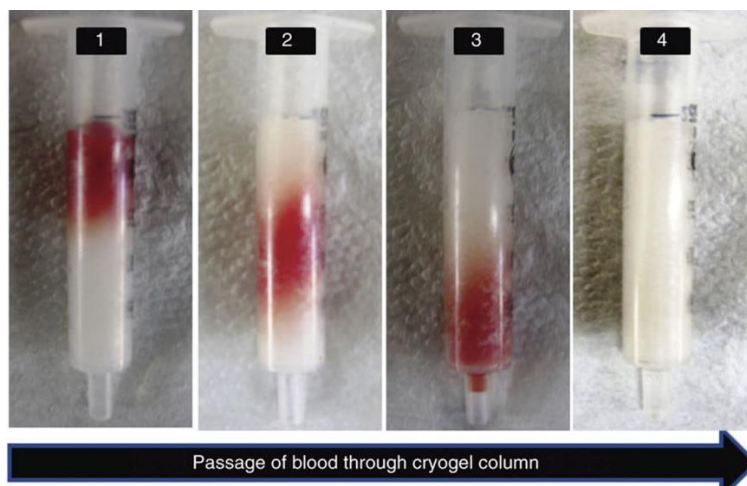
weight limit (NMWL) and will be run in tangential flow in a Cogent Process-Scale Tangential Flow Filtration System. This filter size will allow RBCs to remain in the retentate while remaining enzyme is eliminated through the permeate. The resuspended RBCs from the previous centrifugation step will be transferred into the diafiltration system by a Quattroflow quaternary diaphragm pump. The enzyme-free retentate will flow back into the original storage system feed tank, and the permeate containing the enzymes will be discarded. While this process occurs, new saline-anticoagulant solution will be continuously added into the system feed tank to maintain the osmolality of the RBC solution equivalent to what is found in biological systems to prevent hemolysis and RBC aggregation. As the diafiltration process is performed, the spent saline-coagulant solution that may contain traces of enzyme will be removed and will be replenished by enzyme-free solution. It is assumed that 5% of the RBCs will be lost due to lysis during the diafiltration step, leading to a 95% recovery of the converted RBCs. The diafiltration step will require approximately 2.5 hours to complete and will be run at 4°C. Cleaning time will be set at two hours.

#### **12.C.6. Cryogel-based Affinity Chromatography**

To separate RBCs successfully converted to type-O from those that did not undergo a successful conversion, cryogel-based affinity chromatography will be used. This technique is used instead of using the typical affinity chromatography approach of having protein A-coated resins to which antibody-selected cells would bind. Traditional affinity chromatography for cell separations has been proven to be harsh on RBCs and induce significant lysis. To minimize lysis and costs associated with having to work with even more RBC transfusion units, the cryogel-based technique was chosen given its success in separating blood cells with less than five percent lysis (shown in Figure 12.C.6.1).<sup>50</sup> Supermacroporous cryogel monoliths can have a pore diameter of up to 100 µm, which enables its application for chromatography of cells of varying size (between 1 and 15 µm) without being mechanically entrapped in the column. Convectively transported cells also do not experience large shear forces because of laminar flow of liquid in the interconnected pores in the monolithic cryogel, which helps prevent mechanically-induced cell lysis.

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<sup>50</sup> Kumar A, Srivastava A. Cell separation using cryogel-based affinity chromatography. *Nature Protocols*. 2010Oct7;5(11):1737–47.



**Figure 12.C.6.1.** Blood passage through a cryogel-based affinity chromatography done in academic research to quantify shearing and lysis. The literature reports less than five percent hemolysis of the total RBC population that was introduced into the column.

The cryogel matrix for the column will be produced from different hydrophilic gel-forming polymers either by polymerization of the appropriate monomers, polyacrylamide and poly(dimethylacrylamide). A cryogel affinity matrix will then be developed by covalent coupling of protein A on the surface of the pore wall, which can capture immunoglobulin (IgG) antibodies or IgG-labeled specific cells. Protein A, which consists of a 40- to 60-kD protein present in the cell wall of *Staphylococcus aureus*, has affinity to the Fc portion of the IgG from a wide range of species. Protein A will be covalently coupled to epoxy-activated cryogel monolith by two-step derivatization using functionalization of epoxy-activated cryogel with ethylenediamine and then crosslinking of protein A using glutaraldehyde.

The protein A-bound monolithic cryogel will be used as a generic affinity adsorbent for specific IgG antibodies against A or B antigens and loaded into the chromatography column. The system will operate at a pressure of 1 atm, a temperature of 4°C, and a linear velocity of 2,000 cm/hr. RBCs that have undergone the enzymatic treatment will then be loaded into the cryogel-protein A column. Cells that still have their A or B antigens will bind to the IgG antibodies bound on the cryogel-protein A matrix. The RBCs that were successfully converted, which have no antigens, will not bind to the antibodies and pass through the column. These cells will be collected into a disposable bioprocess container, in which the RBCs will be resuspended to a working volume of 100 L with anticoagulant-saline solution, until the next

centrifugation step. The RBCs will be placed on a WAVE bioreactor rocker table for gentle agitation to prevent them from settling and aggregating.

Mechanical stress will then be applied to the cryogel to release cells that were bound to the column. This system has the advantage though that the mechanical stress only releases bound cells and not the antibodies bound to the protein A, allowing it to be re-used several times before the antibodies begin to lose their binding affinity. This circumvents another issue with using traditional resin: the costly replenishment of antibodies used for binding.

This column will operate for 4.51 hours at a linear velocity of 300 cm/hr. Cleansing and regeneration of the resin will take 1.62 hours.

#### **12.C.7. Microfiltration/Diafiltration**

An additional ultrafiltration/diafiltration step will be implemented after the cryogel-based affinity chromatography step to remove the column buffer components from the final treated RBC product suspension. Pellicone 3 Durapore PVDF microfiltration filters will be used with a 500 kD nominal molecular weight limit (NMWL) and will be run in tangential flow in a Cogent Process-Scale Tangential Flow Filtration System. This filter size will allow RBCs to remain in the retentate while all buffer components are eliminated through the permeate. The resuspended RBCs from the previous centrifugation step will be transferred into the diafiltration system by a Quattroflow quaternary diaphragm pump. The buffer-free retentate will flow back into the original storage system feed tank, and the permeate containing buffer will be discarded. While this process occurs, new saline-anticoagulant solution will be replenished to maintain physiological osmolality for the RBCs. It is assumed that 5% of the RBCs will be lost due to lysis during the diafiltration step, leading to a 95% recovery of the converted RBCs. The diafiltration step will require approximately 2.5 hours to complete and will be run at 4°C. CIP/SIP will take two hours.

#### **12.C.8. Centrifugation**

After the converted RBCs have been separated from non-converted RBCs, the collected contents from the previous microfiltration/diafiltration step will be transferred to a Culturefuge 100 disk stack centrifuge via a Quattroflow quaternary diaphragm pump. The centrifuge will operate at 100 L/hr and at 4°C. The spent anticoagulant-saline solution will be charged to a waste tank for safe disposal. The remaining solid RBC mass will be resuspended in 191 L of Adsol-1

additive solution and transferred into a disposable bioprocess container. Centrifugation will last for two hours to handle the entire blood volume but at lower velocities to prevent shearing. CIP/SIP procedures will require 2.8 hours.

#### **12.C.9. Holding Tank**

Upon completion of centrifugation of the contents that were processed in the microfiltration/diafiltration step, the remaining solid RBC mass will be transferred into a 500 L HyPerforma Smartainer 3D bioprocess container and resuspended in 191 L of Adsol-1 additive solution. It is assumed that the RBC sludge from the centrifugation process will make up approximately 50% of the resuspended solution that will be packaged. The final RBC suspension will have a 50% hematocrit (volumetric ratio of RBCs to total volume). The stored RBC solution will be kept at 4°C and placed on a gentle rocker at 25 rpm to prevent RBCs from settling until final packaging.

#### **12.C.10. Preparation of Converted Red Blood Cell Transfusion Units**

The hyperbaric chamber will be operated in reverse to create pressure driven flow from the storage vessel to dry, empty blood bags connected to the central tubing line. The final output of the RBC treatment process will be prepared as a ready-to-administer product. The converted RBCs stored in the Adsol-1 additive solution will be packaged into TERUFLEX dry blood bags with a working volume of 300 mL at a final concentration of 50% hematocrit. Each batch of the treatment process will produce 1,283 transfusion units of ECO RBCs. The bags will be filled after five pressurization runs, taking a total 1.62 hours to complete packaging. A maximum of 2.5 hours, however, has been scheduled to account for the time needed to fit empty blood bags to the central lines through the RBC suspension will travel. All filled transfusions units of enzymatically converted RBCs will be stored at 4°C at all times and have a maximum shelf-life of 42 days. Note that there will be some variability in the shelf-life depending on the age of the donor RBCs.



# **Section 13:**

## **Energy Balances and Utilities**

### **Requirements**

### **13. Energy Balances and Utilities Requirements**

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Energy balances not applicable.

For utilities requirements, please see section 18.

# **Section 14:**

## **Equipment List and Unit**

### **Descriptions**

## **14. Equipment List and Unit Descriptions**

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### **14.1. Common Units**

#### **14.1.1. Peristaltic Pumps**

Peristaltic pumps will be used to transfer all fluids that contain *E. coli* cell mass or enzyme product. These pumps are best for transferring viscous fluids such as cell suspensions and can show better performances for fluid transport than centrifugal pumps under these operating conditions. The peristaltic pumps will also handle all buffer transfers in the chromatography column steps. Thirty-nine Masterflex (Model 77924-80) 600-rpm pumps will be purchased from Cole-Parmer for \$3,635 per pump. This value includes spares as well. The construction material for each pump is stainless steel AISI 304. Each pump comes equipped with graphical LCD that show four operating modes: continuous run, timed dispense, copy dispense, volume dispense. The flow rate through each pump can vary between 0.001 and 3,400 mL/min with L/S tubing. The sterile L/S tubing will also be provided by Cole-Parmer. Each pump will operate at room temperature and at a pressure drop of 3.5 atm. The power requirement is 253 W. The CIP and SIP processes can operate at a maximum temperature of 90 and 130°C degrees, respectively.

For large volumes of chromatography buffer, primarily in the steps in which ten column volumes must be passed through the unit, Watson Marlow 840 Series Hygienic Peristaltic Pumps will be used. One unit was purchased for each chromatography column required in the facility. Each one of these pumps will operate at room temperature and at a pressure drop of 3.5 atm. GORE® STA-PURE® PFL tubing will be used here to ensure that the tubing can handle all organic solvents and materials found in the buffers. These pumps are mounted on a steel frame with fixed speed motor gearboxes that require a power input of 0.2 W for operation.

#### **14.1.2. Quattroflow Quaternary Diaphragm Pumps**

For the treatment of RBCs, nine Quattroflow quaternary diaphragm pumps (Model QF1200S-CV) will be purchased for \$10,772 per pump from PSG Dover. The Quattroflow pumps are made of stainless steel AISI 304. These pumps were chosen due to their ability to mechanically imitate the pumping of the heart. Each stroke of the 4-piston diaphragm is generated by an eccentric shaft connected to an electric motor. The method of operation of the Quattroflow pumps

allows them to transfer shear-sensitive biological molecules and cells with minimal impact, which helps prevent additional cell lysis that could affect the RBC production goal. The flow rate through each pump can vary between 10 and 1,200 L/hr with a 5° eccentric shaft. Each Quattroflow pump will be operated at 800 rpm to achieve a flow rate of 350 L/hr and at a pressure drop of 6 atm. For sterile sealing, all valves and O-rings are sealed with ethylene propylene diene monomer rubber. The power requirement for each Quattroflow pump is 370 W. The CIP and SIP processes can operate at a maximum temperature of 90 and 130°C degrees, respectively.

#### **14.1.3. Media Sterilization Filters**

0.2 µm pore-sized Millipore Express® SHF filters will be used to purify bacterial culture media by removing any impurities and toxins before introduction in the stainless steel fermenters. Each filter has a nominal length of 30 inches, a diameter of 6.9 cm, and a filtration area of 1.62 m<sup>2</sup>. The device uses polyethersulfone (PES) for the filtration chemistry. The device material is polypropylene/polysulfone. The seal material is code 7 silicone. All filtration cartridges were sterilized by 25 forward or 22 forward and 3 reverse SIP cycles of 30 min at 135 °C. Sterilization can also be done using 25 autoclave cycles of 60 min at 126 °C. The maximum forward inlet pressure is 7 bar and the maximum reverse inlet pressure is 2.07 bar, both at 25°C. The maximum forward pressure differential is 6.9 bar and the maximum reverse pressure differential is 2.1, both at 25°C. Each filter costs \$650/unit. The filters require a type of housing for security, which will be contracted to Millipore for a one-time cost of \$2,000. The filters are disposable and replaced after every batch. Since they are disposable, no CIP or SIP will be required for the filter. SIP, however, is required for the housing unit.

#### **14.1.4. Digital Control Units**

Digital control units will be purchased to conduct proportional integral derivative (PID) feedback control. The PID controllers, purchased from Omega, will be used to control the agitation speed and maintain temperature at 37°C, pH between 7.0 and 7.2, constant percent oxygen at 40%, and a constant liquid level to avoid excessive foaming for all bioreactor systems in the *E. coli* BL21(DE3) upstream culture process. A total of 30 units will be purchased, including back-ups. Each unit will be purchased at a price of \$300/unit.

## **14.2. Upstream Process for *E. coli* Growth**

### **14.2.1. Inoculum Growth Bioreactor**

The ambr 250 modular bioreactor system will be purchased from Sartorius at \$45,799 for the control tower and system. Each individual vessel will be purchased for \$155. The bioreactor tower and control system is made of stainless steel, while the bioreactor vessel is sterile plastic. Each batch will begin with a new, sterile ambr 250<sup>®</sup> bioreactor with a freshly thawed vial of *E. coli* BL221(DE3) cells. All bioreactor vessels are single-use and disposable. Agitation will occur at 300 rpm using a dual 20 mm Rushton impeller. The ratio of the impeller diameter to the bioreactor inner diameter is 0.5. The geometric ratio of height to inner diameter of the reactor vessel is 1.8. The bioreactor vessel has a maximum working volume of 0.250 L; the chosen working volume for this unit operation is 0.110 L. Temperature control will be done using electrical heating. The vessel design incorporates disposables sensors to simplify system preparation. The bioreactor package includes DO sensors to measure pO<sub>2</sub>, disposable pH electrodes, integrated gas and liquid inlet filters, and an integrated condenser for output to exhaust gas analysis. The bioreactor will operate at a temperature of 37°C, a pressure of 1.0 atm, a pH of 7.0-7.2, and 40% pO<sub>2</sub>. Each vessel will be in operation, preferably overnight, for 17.68 hours, regardless of the enzyme product. It is assumed that 5% of the bacterial population will die during culture, due to shear-induced lysis or health complications, leading to a percent yield of 95%.

### **14.2.2. Growth Bioreactor**

The cell suspension from the inoculum growth reactor will be transferred into a larger bioreactor to continue cell growth in a larger culture volume to prepare for inoculation of the final production fermenters. The growth bioreactor system will consist of a stainless steel AISI 316L BIOSTAT<sup>®</sup> B control tower and an autoclavable borosilicate glass vessel. It also allows for four peristaltic pumps integrated into the control unit, two external peristaltic pumps, and two balances connections. The complete bioreactor system will be purchased for \$100,000. The bioreactor vessel has a maximum working volume capacity of 10 L; the chosen working volume for this process is 5.44 L. The dimensions of the entire system are 330mm x 860mm x 420 mm. Agitation will be performed at 800 rpm with a 6-blade disk Rushton turbine impeller to allow for an volumetric oxygen transfer coefficient greater than 40 hr<sup>-1</sup>. Temperature control will be done using

a single-wall heated jacket. The bioreactor will operate at a temperature of 37°C, a pressure of 1.0 atm, a pH of 7.0-7.2, and 40% pO<sub>2</sub>. Each vessel will be in operation for 24.03 hours for α-N-acetylgalactosaminidase or for 23.69 hours for α-galactosidase. Standard CIP procedures will be used for sterilization of control tower when necessary. The borosilicate glass vessel will be autoclaved at 121°C for one hour. It is assumed that 5% of the bacterial population will die during culture, due to shear-induced lysis or health complications, leading to a percent yield of 95.5%.

#### **14.2.3. Production Bioreactors**

The cell suspension from the growth bioreactor will be split between two stainless steel (AISI 304) fermenters. The BIOSTAT® D-DCU, which includes the control tower and the two fermenters, will be purchased from Sartorius for a total of \$300,000. The bioreactor vessel has a maximum working volume capacity of 200 L; the chosen working volume for this process is 198 L for each reactor. The dimensions of the entire system are 3.1m x 3.05m x 1.8m. Agitation will be performed at 800 rpm with a 6-blade disk Rushton turbine impeller to allow for an volumetric oxygen transfer coefficient of 308 hr<sup>-1</sup>. The ratio of the impeller diameter to the bioreactor inner diameter is 0.5. The geometric ratio of height to inner diameter of the reactor vessel is 3.0. The high performance servo drive motor assembly combines low shear, gentle agitation for cell cultures and high speed mixing for microbial high cell density cultivation, ensuring high oxygen transfer rates. Temperature control will be done using an integrated closed loop system with a recirculation pump and heat exchanger. The bioreactors will operate at a temperature of 37°C (for growth) and 25°C (for protein expression), a pressure of 1.0 atm, a pH of 7.0, and 40% pO<sub>2</sub>. Each vessel will be in operation for 25.89 hours for cell growth and then an additional five hours for enzyme protein expression. Standard CIP and SIP procedures will be used for sterilization. It is assumed that 5% of the bacterial population will die during culture, due to shear-induced lysis or cell viability complications, leading to a percent yield of 95.5%.

#### **14.2.4. Cell Culture Storage Vessel**

The cell suspension from the production fermenters will be stored in a Thermo Fisher 500L HyPerform Single-Use Mixer, which has a purchase cost of \$165,000. Each disposable bag is appraised at \$300. The mixer is made of stainless steel AISI 304, while the single-use disposable biocontainers will be made of CX5-14 multi-layer plastic. The dimensions of the unit are 122cm

x 129cm x 198cm. The unit will be operated at a pressure of 0.03 atm and a temperature of 5°C. The liquid chill-down time for the mixer is 3.7 hours.

### **14.3. Downstream Purification Section**

#### **14.3.1. Disk stack centrifuge**

The purpose of the centrifuge is to separate the solid biomass (*E. coli* cells with desired enzyme product in their cytoplasm) from the spent liquid media. All spent culture media will be directed to a waste holding tank while the solid *E. coli* cell pellet will be moved downstream to harvest the  $\alpha$ -galactosidase or  $\alpha$ -N-acetylgalactosaminidase enzyme product. A Culturefuge 100 disk stack centrifuge will be purchased from Alfa Laval for \$46,500 per unit. The solid biomass product will be in the form of a cell sludge that is 40% by mass solids and 60% by mass water. The centrifuge unit will consist of a molybdenum-bearing austenitic stainless steel bowl and a cast iron grey bottom frame. The centrifuge has a maximum operating capacity of 2,000 L/hr, 12,220 g for G-force, and 9,550 rpm bowl speed. The volumetric flow rate that the Culturefuge 100 will operate at is 100 L/hr. The unit will operate at a temperature of 25°C and a pressure of 1.0 atm. The process time, including CIP and SIP procedures, will take approximately 6.8 hours. It is assumed that approximately five percent of product enzyme will be lost in this unit operation.

#### **14.3.2. High Pressure Homogenization**

The cell sludge from the centrifugation step will be sent through a M175 Aseptic Microfluidizer, purchased from Microfluidics, to disrupt the cell membrane of the *E. coli* bacterial cells and release the internalized enzyme product. The device can operate at a maximum pressure of 30,000 psi with a volumetric flow rate of 7.56 L/min. The hydraulic capacity of the fluidizer is 13 L/hr. The hold-up volume is less than one liter. The microfluidizer comes equipped with a heat exchanger for product temperature control, using a pharma grade, double tubesheet style. For cooling purposes, a chilled water loop will be used. The unit also comes equipped with a feed pump for air and requires a flow rate of 0.65 m<sup>3</sup>/min at 6.2 atm. For product pathing cooling and dry down, a sterile compressed air stream of 0.085 m<sup>3</sup>/min at 1 atm is required. The overall power requirement for this device is 18.6 kW. The cell membrane disruption process via



microfluidization will require approximately four hours. CIP and SIP procedures will last two hours. Steam-in-place requirements are 22.6 kg/hr at 2.4 atm.

The microfluidizer will pump the sludge through a pressure differential of 20,000 psi, and the cells will pass through fixed-geometry microchannels to induce high linear velocities. The high velocities at which the cells travel generate impact and shear-inducing conditions to lyse the cells. The shearing conditions are not sufficient to denature proteins, providing an effective method to collect cytoplasmic protein product. It is assumed that product recovery is approximately 95%.

#### **14.3.3. Microfluidization Product Resuspension Container**

After centrifugation and homogenization, the resulting cell sludge with product will be resuspended in equilibration buffer to a volume equal to the volume of culture media in which the cells were originally suspended. This amount is added to ensure that the pH of the resulting suspension is that the same as that of the equilibration buffer, a pH of 7.4. The cell sludge will be resuspended in a HyPerforma Single-Use Mixer lined with a 500 L 3D bioprocess container, both purchased from Thermo Fisher Scientific. The mixer unit is made of stainless steel AISI 304. The disposable containers are sterile single-use CX5-14 multi-layer plastic. A volume equal to that of the cell sludge will be first added to the mixer, after which the cell cludge will be pumped into the device via peristaltic pump. Equilibration buffer will then be added until the pH and volumetric conditions are satisfied. The HyPerforma Single-Use Mixer will be purchased at \$150,000 per unit, while each 3D bioprocess container will be purchased for \$300/ per bag. A PID feedback controller will be incorporated into this step to maintain the agitation speed of the mixer at 300 rpm, the temperature at 25°C, and the pressure at 0.003 atm. The process time, including CIP and SIP procedures, will take approximately 3.6 hours. It is assumed that 5% of product enzyme will be lost in this unit operation.

#### **14.3.4. Cobalt Affinity Chromatography Column**

A cobalt affinity chromatography system will be used as a first purification pass to separate the enzyme product from cell debris and intracellular materials. A Resolute 280 chromatography column, from Pall Corporation, will be purchased at \$110,000 per unit. The column is comprised of electropolished stainless steel and has a diameter of 280 mm. It will be packed with HisPur Cobalt Resin, which contains a cobalt-chelate coordination core to bind His<sub>6</sub>-tagged proteins, and

is applicable for treatment of both enzymes. The resin has a void fraction of 0.3. Each column is designed to separate either 205 grams of  $\alpha$ -N-acetylgalactosaminidase or 189 grams of  $\alpha$ -galactosidase. 10.25 L of resin will be required to purify  $\alpha$ -N-acetylgalactosaminidase, and 9.45 L will be needed for purification of  $\alpha$ -galactosidase. The required height for the column is 237 mm. The column volume required to satisfy both resin volume requirements is 14.6 L. The resin allows for an operating linear velocity of 150 cm/hr and has a binding capacity of 20 grams of proteins per liter of resin. The resin can be used 25 times before losing efficiency and requiring replacement. The column will be located in a cold room section of the work space so that operating conditions are 4°C and 1.0 atm. The entire column operating time, including regeneration and sterilization procedures, is 7.46 hours. A product recovery of 95% is assumed.

#### **14.3.5. Cobalt Affinity Chromatography Collection Container**

All column flow-through will be directed to a waste tank for heat inactivation to eliminate any remaining active bacterial cells. The eluted product from the column will be collected in a separate single-use 200 L 3D Productainer bioprocess bag purchased from Thermo Fisher Scientific at \$250/bag. These disposable containers are sterile single-use CX5-14 multi-layer plastic. The working volume of each container will be 147 L for  $\alpha$ -galactosidase elute and 94.5 L for  $\alpha$ -N-acetylgalactosaminidase elute. The containers will be stored at 4°C if the following chromatography column is not immediately available for use.

#### **14.3.6. Hydrophobic Interaction Chromatography Column**

A hydrophobic interaction chromatography step will be used to separate endotoxins from the  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase products to comply with FDA standards. A Resolute 800 chromatography column, from Pall Corporation, will be purchased for \$250,000. The column is comprised of electropolished stainless steel and has a diameter of 800 mm. It will be packed with POROS Benzyl HIC Resin, which contains beads with aromatic hydrophobic benzyl ligand that strongly bind the highly hydrophobic endotoxins. The resin has a void fraction of 0.4. Each batch will separate either 11.5 grams of endotoxin from the  $\alpha$ -N-acetylgalactosaminidase culture or 10.6 grams from the  $\alpha$ -galactosidase culture. 57.5 L of resin will be required to purify  $\alpha$ -N-acetylgalactosaminidase, and 52.9 L will be needed for purification of  $\alpha$ -galactosidase. The required height for the column is 191 mm. The column volume required

to satisfy both resin volume requirements is 95.9 L. The resin allows for an operating linear velocity of 150 cm/hr and has a binding capacity of  $2 \times 10^6$  endotoxin units/mL resin. The resin can be used ten times before losing efficiency and requiring replacement. The product purity, according to the manufacturer's protocol, is  $\geq 90\%$ . The column will be located in a cold room section of the work space so that operating conditions are 4°C and 1.0 atm. The entire column operating time, including regeneration and sterilization procedures, is 4.68 hours. A product recovery of 95% and an endotoxin removal of 90% are assumed.

#### **14.3.7. Hydrophobic Interaction Chromatography Collection Container**

All column flow-through will be directed to a waste tank for heat inactivation to eliminate any possible remaining active bacterial cells. The eluted product will be collected in a separate single-use 1500 L 3D Labtainer bioprocess container purchased from Thermo Fisher Scientific at \$500/bag. These disposable containers are sterile single-use CX5-14 multi-layer plastic. The container will be stored at 4°C if the following chromatography step is not immediately available for use.

#### **14.3.8. Endotoxin Affinity Chromatography Column**

An endotoxin affinity chromatography system will be used to separate endotoxins from the  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase enzyme products. A Resolute 280 chromatography column, from Pall Corporation, will be purchased at \$110,000 per unit. It is comprised of electropolished stainless steel and has a diameter of 280 mm. This column will be packed with Pierce High-Capacity Endotoxin Removal Resin, which contains poly( $\epsilon$ -lysine) coated beads that bind endotoxins. The resin has a void fraction of 0.4. Each batch will separate either 1.15 grams of endotoxin from the  $\alpha$ -N-acetylgalactosaminidase culture or 1.06 grams from the  $\alpha$ -galactosidase culture. 5.75 L of resin will be required to purify  $\alpha$ -galactosidase, and 5.29 L will be needed for purification of  $\alpha$ -N-acetylgalactosaminidase. The required height for the column is 156 mm. The column volume required to satisfy both resin volume requirements is 9.59 L. The resin allows for an operating linear velocity of 150 cm/hr and has a binding capacity of  $2 \times 10^6$  endotoxin units/mL resin. The resin can be used five times before losing efficiency and requiring replacement. The product purity, according to the manufacturer's protocol, is  $\geq 90\%$ . The column will be located in a cold room section of the work space so that operating conditions are 4°C and

1.0 atm. The entire column operating time, including regeneration and sterilization procedures, is 3.01 hours. A product recovery of 95% and an endotoxin removal of 99% are assumed.

#### **14.3.9. Endotoxin Affinity Chromatography Collection Container**

All additional column flow-through will be directed to a waste tank for heat inactivation to eliminate any possible remaining active bacterial cells. The eluted product from the  $\alpha$ -galactosidase separation will be collected and stored in a single-use 50 L 2D Productainer bioprocess bag purchased from Thermo Fisher Scientific at \$37.54/bag. These disposable containers are sterile single-use CX5-14 multi-layer plastic. The containers will be stored at 4°C if the following chromatography column is not immediately available for use.

#### **14.3.10. Ultrafiltration/Diafiltration Unit**

An ultrafiltration/diafiltration process will be implemented using a Cogent M1 Process Scale Tangential Flow Filtration System. It is manufactured by EMD Millipore and has a purchase cost of \$47,999. The system will be equipped with Pellicon 3 Ultracel filter cassettes made of regenerated cellulose and polyethylene. The filtration area of the cassettes is 1.14m<sup>2</sup> and will handle a flow rate of 4 L/min. The system can operate with a maximum pressure drop of 2 atm. The stainless steel cassette holder will be purchased at \$995. A pack of 100 Ultracel membranes will also be purchased for \$479.08. The system will be operated in a cold room at 4°C and 1 atm.

#### **14.3.11. Bulk Microfiltration Unit**

A bulk microfiltration pack will be purchased from EMD Millipore for \$503 for a pack of three filters. The filters will be hydrophobic Durapore PVDF membranes with a 0.22  $\mu$ m pore diameter size. Given that there is two-fold difference in hydrophobicity between endotoxins and the ECO-zymes, the use of bulk microfiltration allows endotoxins to be retained in the filter while enzyme product is filtered. The enzyme product will go through two passes of bulk microfiltration to guarantee compliance of FDA standards regarding endotoxin concentration. It is assumed that these filters remove 99% of endotoxins, as stated in the manufacturer's protocol. The diameter and thickness of the filter are 142 mm and 125  $\mu$ m, respectively. The PVDF filters and their respective holders are disposable, and one of each will be used per batch. The filters have a 100 cm<sup>2</sup> surface area and will run at a flowrate of 16.5 L/min. The porosity of the filters is 75%. Protein binding at

the membrane is 150  $\mu\text{g}/\text{cm}^2$ . Recovery of ECO-zyme product in this step is 95% for each pass. All microfiltration units are single-use and disposable once the batch process has completed.

#### **14.3.12. Final Product Holding Container**

The final concentrated product from the bulk microfiltration step will be concentrated to 1 mg enzyme/mL and stored in a five 50 L 2D Labtainer bioprocess bags purchased from Thermo Scientific for \$37.54/bag. The bioprocess container is made of sterile single-use CX5-14 multi-layer plastic. Containers will be stored at 4°C in a cold room if needed in the immediate future for RBC conversion treatment. Otherwise, bags will be kept at -20°C for long-term storage.

### **14.4. RBC Conversion Treatment Process**

#### **14.4.1. Hyperbaric Chamber**

A hyperbaric chamber will be used to empty 264 blood bags for every pressurization run into a storage container. The chamber will be designed to sustain preferential differentials up to 1.5 atm. A total of 1,973 blood bags are expected to be processed, on average, for every batch. Each batch treatment will take approximately 2.5 hours. The process will be carried out in a cold room at 4°C.

#### **14.4.2. Hyperbaric Chamber Collection Container**

The RBCs collected from donor transfusion units will be emptied by the hyperbaric chamber into three 200 L 3D Productainer bioprocess bags, purchased from Thermo Fisher Scientific at \$250/bag, all of which are sterile single-use CX5-14 multi-layer plastic containers. The RBCs will be kept at 4°C until proceeding to the centrifugation step. They will also be kept on a rocker to prevent the cells from settling and to maximize the RBC content drained from the containers.

#### **14.4.3. Disk Stack Centrifuge**

Another Culturefuge 100 disk stack centrifuge, purchased from Alfa Laval for \$46,500 per unit, will be used to separate RBCs from their spent additive preservation solution. Upon centrifugation, the solid RBCs will settle to the bottom of the centrifuge bowl. All spent

preservation fluid will be directed to a biowaste holding tank for heat inactivation while the RBC pellet will be directed to a WAVE bioreactor. The centrifuge unit will consist of a molybdenum-bearing austenitic stainless steel bowl and a cast iron grey bottom frame. The centrifuge has a maximum operating capacity of 2,000 L/hr, 12,220 g for G-force, and 9,550 rpm bowl speed. The volumetric flow rate that the Culturefuge 100 will operate at is 100 L/hr. The unit will operate at a temperature of 4°C and a pressure of 1.0 atm. The process time, including CIP and SIP procedures, will take approximately 4.8 hours. It is assumed that 5% of product enzyme will be lost in this unit operation.

#### **14.4.4. WAVE Bioreactor**

The RBCs to be converted will be transferred from the disposable holding bag into five WAVE bioreactors. The chosen model is the BIOSTAT<sup>®</sup> RM bioreactor system, which includes the control tower and the disposable bag rocker. Each system will cost \$20,000. Additionally, the disposable bag necessary is the FLEXSAFE<sup>®</sup> RM. The control tower is made of stainless steel AISI 304; the rocker, stainless steel ABS; and the disposable bags, polyethylene multilayer plastic. The area dimensions of each individual system is 740 mm x 720mm. The temperature control consists of an integrated open thermostat system with a circulation pump and an automatic cooling water valve. Temperature can be increased and decreased in intervals of 0.5°C every 45 seconds. The power requirements are 1.20 kW per unit. Each WAVE bioreactor will operate at a temperature of 25°C, a pressure of 1.0 atm, a pH of 7.2 to 7.4, and 40% pO<sub>2</sub>. Each bag has a total volume of 200 L but only has a working capacity of 100 L, which is what approximately used in each. The rocking base will perform 15 rocks/min at an angle of 10°.

#### **14.4.5. Disk Stack Centrifuge**

Another Culturefuge 100 disk stack centrifuge, purchased from Alfa Laval for \$46,500 per unit, will be used to separate the converted RBCs from the enzyme in solution. Upon centrifugation, the solid RBCs will settle to the bottom of the centrifuge bowl while the  $\alpha$ -galactosidase or  $\alpha$ -N-acetylgalactosaminidase enzyme will remain in solution. All spent fluid with enzyme will be directed to a waste holding tank while the RBC pellet will be collected into a bioprocess container until sent transferred into the diafiltration unit. The centrifuge unit will consist of a molybdenum-bearing austenitic stainless steel bowl and a cast iron grey bottom frame.

The centrifuge has a maximum operating capacity of 2,000 L/hr, 12,220 g for G-force, and 9,550 rpm bowl speed. The volumetric flow rate that the Culturefuge 100 will operate at is 100 L/hr. The unit will operate at a temperature of 4°C and a pressure of 1.0 atm. The process time, including CIP and SIP procedures, will take approximately 4.8 hours. It is assumed that 5% of product enzyme will be lost in this unit operation.

#### **14.4.6. Diafiltration Unit**

A diafiltration unit operation will be implemented to remove ECO-zyme from the RBC suspension. The unit operation will be done using a Cogent Process Scale Tangential Flow Filtration System from EMD Millipore, with a purchase cost of \$47,999. The system will implement Pellicone 3 Durapore PVDF Ultracel microfiltration filters will be used with a 500 kD NMWL, which is large enough for all enzymes to pass through but retain all RBCs. Each pack of filters has a purchase cost of \$479.08, while the sterile stainless steel cassette holder will cost \$995. The filters will be made of regenerated cellulose with a polyethylene frame. The filtration area of each filter is 1.14 m<sup>2</sup> and will be operated at 4 L/min. It is assumed that 95% the treated RBCs will be recovered in this step, with a 5% loss associated with filtration through the cassette membrane or lysis. This entire process will be done at 4°C in a cold room and at a pressure of 1 atm.

#### **14.4.7. Cryogel-based Affinity Chromatography Column**

A cryogel-based affinity chromatography column will be used to separate non-converted RBCs from the successfully converted universal RBCs. A Resolute 280 chromatography column, from Pall Corporation, will be purchased for \$200,000. The column is comprised of electropolished stainless steel and has a diameter of 280 mm. It will be packed with a cryogel polymer made of polyacrylamide and poly(dimethylacrylamide) that is coated in protein A-IgG. The protein A binds IgG antibodies that will target blood group antigens on RBCs. The cryogel will be pre-loaded with 72.1 mg of blood group-specific IgG antibody per batch prior to running RBCs through the column. Each column run will process 1,237 L of processed blood. The resin has a void fraction of 0.4. For running the column, 14.42 L of cryogel will be needed for purification processes. The cryogel will be used five times before replenishing and antibody replacement. The required height for the column is 292 mm. The column volume required to satisfy the cryogel volume requirement is 18.0 L. The cryogel allows for an maximum linear velocity of

1,200 cm/hr but will operated at 300 cm/hr. The product purity is reported to be  $\geq 95\%$ . The column will be located in a cold room section of the work space so that operating conditions are 4°C and 1.0 atm. The entire column operating time, including regeneration and sterilization procedures, is 4.51 hours. A product recovery of 95% is assumed.

#### **14.4.8. Disk Stack Centrifugation**

A Culturefuge 100 disk stack centrifuge, purchased from Alfa Laval for \$46,500 per unit, will separate the converted RBCs from any remaining buffer solution. It is assumed that the output of the centrifuge will be 60% by mass water and 40% by mass RBCs. All spent buffer and solution will be directed to a waste holding tank while the RBCs will be collected into a final product holding container. The centrifuge unit will consist of a molybdenum-bearing austenitic stainless steel bowl and a cast iron grey bottom frame. The centrifuge has a maximum operating capacity of 2,000 L/hr, 12,220 g for G-force, and 9,550 rpm bowl speed. The volumetric flow rate that the Culturefuge 100 will operate at is 100 L/hr. The unit will operate at a temperature of 4°C and a pressure of 1.0 atm. The process time, including CIP and SIP procedures, will take approximately 4.8 hours. It is assumed that 5% of product enzyme will be lost in this unit operation.

#### **14.4.9. Final Product Holding Container**

After centrifugation, the converted RBCs will be transferred into a 500 L HyPerforma Smartainer 3D bioprocess container made of sterile single-use CX5-14 multi-layer plastic. The RBC mass will be resuspended to a volume of 191 L of Adsol-1 additive preservation solution. The RBCs will be kept at 4°C from this point forward to preserve their biological activity. The container will be placed on a WAVE rocker set at an agitation speed of 25 rpm to prevent RBCs from settling until final packaging.



# **Section 15:**

# **Specification Sheets**

## **15. Specification Sheets**

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The following pages contain the specification sheets for all unit operations. Description and function, vendor, operation, characteristics, operating conditions, and purchase cost are all detailed.

## 15. 1. Inoculum Bioreactor (P-1, PFD#1)

Description and Function Inoculum for the growth bioreactor will be prepared and grown in a 0.250 L single-use, stirred tank bioreactor, with a working volume of 110 mL. The purchased unit will also include a control tower to monitor aeration, pH, temperature, and rocking speed. The vessels are made of single layer injection molded plastic and are 15 kGy  $\beta$ -irradiated for sterilization.

Vendor Sartorius Stedim Biotechnology Group

Operation Batch

<u>Materials Handled</u>	<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
Cells	$5.60 \times 10^{-7}$	$4.87 \times 10^{-5}$
Nutrients	$9.74 \times 10^{-5}$	0
Water	0.110	0.105
Kanamycin	$5.50 \times 10^{-6}$	$5.50 \times 10^{-6}$
Chloramphenicol	$3.40 \times 10^{-6}$	$3.40 \times 10^{-6}$
Endotoxin	0	$3.20 \times 10^{-3}$
Waste	0	$3.39 \times 10^{-4}$

<u>Characteristics</u>	Model:	ambr <sup>®</sup> 250 modular system
	Material construction:	Sterile plastic vessel, stainless steel control tower
	Heating method:	Electric heating
	Percent yield:	95%
	Sterilization:	Disposable stirred tank
	Sparger:	Microsparger
	Agitation speed:	300 rpm
	Impeller configuration:	Dual 20 mm Rushton impeller
	Ratio d:D <sub>i</sub>	0.5
	Ratio H:D <sub>i</sub>	1.8
	Volume:	0.2 L

<u>Operating Conditions</u>	Temperature:	37°C
	Pressure:	1.0 atm
	pH:	7.0-7.2
	pO <sub>2</sub> :	40%
	Growth time:	17.86 hours

<u>Purchase Cost</u>	Control tower:	\$45,799
	Bioreactor vessel:	\$155/vessel

## 15.2. Growth Bioreactor (P-2, PFD#1)

<u>Description and Function</u>	The growth bioreactor will consist of an autoclavable glass vessel operated by a control tower. It will be used to further increase the cell concentration before splitting the culture between two stainless steel fermenters. The total reactor volume is 10L and will operate with a working volume of 5.44 L. The PID controller, ez-Control from Applikon, will be purchased separately to monitor and control the pH, temperature, pO <sub>2</sub> , foam level, glucose and agitation rate.	
<u>Vendor</u>	Sartorius Stedim Biotechnology Group	
<u>Operation</u>	Batch	
<u>Materials Handled</u>	<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Cells	$4.62 \times 10^{-5}$
	Nutrients	$3.76 \times 10^{-2}$
	Water	5.44
	Kanamycin	$2.72 \times 10^{-4}$
	Chloramphenicol	$1.85 \times 10^{-4}$
	Endotoxin	$3.20 \times 10^{-3}$
	Waste	0
		1.88 $\times 10^{-2}$
		0
		5.21
		$2.72 \times 10^{-4}$
		$1.85 \times 10^{-4}$
		0.158
		0.584
<u>Characteristics</u>	Model:	BIOSTAT® B
	Material Construction:	Borosilicate glass vessel, stainless steel AISI 316L control tower
	Dimensions:	330mm x 860mm x 420mm
	Heating method:	Single-wall heated jacket
	Percent Yield:	95%
	Sterilization:	CIP/SIP
	Agitation speed:	800 rpm
	Impeller configuration:	Dual 20 mm Rushton impeller
	Volume:	10 L
<u>Operating Conditions</u>	Temperature:	37°C
	Pressure:	1.0 atm
	pH:	7.0
	pO <sub>2</sub> :	40%
	Growth Time:	24.03 hr for $\alpha$ -galactosidase, 23.69 hr for $\alpha$ -N-acetylgalactosaminidase
<u>Purchase Cost</u>	Bioreactor system:	\$100,000
	Controller:	\$500

### 15.3. Production Bioreactors (P-3, P-4; PFD#1)

<u>Description and Function</u>	The production bioreactor system will consist of two stainless steel bioreactors with a volume of 323 L and a maximum working volume of 200 L. Each reactor will handle a working volume of 198 L and will be used to grow up cells to a desired cell concentration and for induce protein production using induction by IPTG. The controller, ez-Control from Applikon, will be purchased separately to monitor and control the pH, temperature, pO <sub>2</sub> , foam level, glucose and agitation rate.		
<u>Vendor</u>	Sartorius Stedim Biotechnology Group		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
(overall)	Cells	1.78×10 <sup>-2</sup>	11.5
	Nutrients	23.1	0
	Water	396	379
	IPTG	9.44×10 <sup>-2</sup>	9.44×10 <sup>-2</sup>
	GalNAc-ase	0	0.216
	OR α-galactosidase	0	0.199
	Endotoxin	0.158	11.5×10 <sup>-3</sup>
	Waste	0	358
<u>Characteristics</u>	Model:	BIOSTAT <sup>®</sup> D-DCU	
(for each reactor)	Material Construction:	Stainless steel AISI 304	
	Dimensions:	3.1m x 3.05m x 1.8m	
	Heating method:	Closed loop system with recirculation pump and heat exchanger for heating and cooling	
	Percent Yield:	95%	
	Sterilization:	CIP/SIP	
	Agitation speed:	800 rpm	
	Impeller configuration:	6-blade disk Rushton turbine	
	Ratio d:D <sub>i</sub>	0.5	
	Ratio H:D <sub>i</sub>	3	
	Maximum working volume:	200 L	
	Total volume:	323 L	
<u>Operating Conditions</u>	Temperature:	37°C (growth), 25°C (production)	
	Pressure:	1.0 atm	
	pH:	7.0	
	pO <sub>2</sub> :	40%	

Growth Time: 25.89 hours  
Protein Production Time: 5 hours

Purchase Cost

Bioreactor: \$150,000/reactor  
Controller: \$500

#### 15.4. Cell Culture Storage Tank (P-6, PFD#1)

<u>Description and Function</u>	Upon completion of fermentation and protein production, the cell suspension with internalized enzyme product from the production bioreactors will be quickly transferred into a 500 L HyPerforma Single-Use Mixer. This will enable CIP and SIP procedures to begin promptly. Additionally, the suspension will be gently agitated to prevent cells from settling.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material construction:	Stainless steel AISI 304, sterile single-use CX5-14 multi-layer plastic
	Dimensions:	122cm x 129cm x 198 cm
	Volume:	500 L
	Sterilization:	Disposable single-use bags, CIP/SIP for mixer when needed
	Liquid chill-down time:	3.7 hours
	Agitation speed:	300 rpm
<u>Operating Conditions</u>	Temperature:	5°C
	Pressure:	0.03 atm
<u>Purchase Cost</u>	\$100,000/mixer \$300/bag	

## 15.5. Disk Stack Centrifuge (P-1, PFD#2)

<u>Description and Function</u>	The Culturefuge 100 disk stack centrifuge will be used to separate the cells (solid biomass) from the spent media (liquid). The resulting cell sludge will be 60% by mass water and 40% by mass solid. Five percent of the cell suspension from the production fermenters are lost due to transfer losses prior to centrifugation.		
<u>Vendor</u>	Alfa Laval		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Cells	11.5	10.9
	Media	379	12.7
	IPTG	9.44×10 <sup>-2</sup>	8.97×10 <sup>-2</sup>
	Kanamycin	2.72×10 <sup>-4</sup>	2.59×10 <sup>-4</sup>
	Chloramphenicol	1.85×10 <sup>-4</sup>	1.76×10 <sup>-4</sup>
	Endotoxin	11.5×10 <sup>-3</sup>	10.9×10 <sup>-3</sup>
	Waste	0	368
	GalNAc-ase	0.205	0.195
	OR α-galactosidase	0.189	0.180
<u>Characteristics</u>	Model:	Culturefuge 100	
	Centrifuge type:	Disk stack	
	Material construction:	Mo-bearing austenitic steel for body and cover; cast grey iron bottom frame	
	Finish:	Electro-polished	
	Hydraulic capacity:	2,000 L/hr capacity	
	G-force:	12,220 g maximum	
	Bowl speed:	9,550 rpm maximum	
	Percent Yield:	95%	
	Sterilization:	CIP/SIP	
<u>Operating Conditions</u>	Temperature:	25°C	
	Pressure:	1.0 atm	
	Flowrate:	200 L/hr	
<u>Purchase Cost</u>	\$46,500/unit		



## 15.6. High Pressure Homogenizer/Microfluidizer (P-3, PFD#2)

<u>Description and Function</u>	The high-pressure M-7125 Microfluidizer is used to homogenize and lyse cells after centrifugation to release expressed α-galactosidase or α-N-acetylgalactosaminidase, along with cell debris and other excess proteins. The microfluidizer generates a pressure differential of 20,000 psi, causing high-velocity pressure-driven flow of cells across fixed-geometry channels. The geometry of the channels combined with the high velocity generate high impact and shearing conditions that disrupt the cell membrane and cause the <i>E. coli</i> cells to lyse.		
<u>Vendor</u>	Microfluidics		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Cells	10.9	10.4
	B-PER	326	0
	Endotoxin	10.9×10 <sup>-3</sup>	10.4×10 <sup>-3</sup>
	Water	12.7	12.1
	IPTG	8.97×10 <sup>-2</sup>	8.52×10 <sup>-2</sup>
	Kanamycin	2.78×10 <sup>-4</sup>	2.64×10 <sup>-4</sup>
	Chloramphenicol	1.85×10 <sup>-4</sup>	1.76×10 <sup>-4</sup>
	GalNAc-ase	0.195	0.185
	OR α-galactosidase	0.180	0.171
	Waste	0	328
<u>Characteristics</u>	Model:	M-7125 Series Microfluidizer	
	Material construction:	Stainless steel	
	Dimensions:	272cm x 161cm x 211cm	
	Hydraulic capacity:	13 L/hr capacity	
	Maximum pressure:	2000 atm	
	Percent yield:	95%	
	Hold-up volume:	<1 L	
	Power requirement:	18.6 kW	
<u>Operating Conditions</u>	Temperature:	25°C	
	Pressure:	1,316 atm	
	Flow rate:	13 L/hr	
<u>Purchase Cost</u>	\$38,740/unit		

### 15.7. Cell Sludge Resuspension Container (P-4, PFD#2)

<u>Description and Function</u>	A 3D bioprocess container will be used to collect the lysed biomass and harvested enzyme product after the microfluidic cell membrane disruption. The biomass and product will be resuspended in equilibration buffer to prepare for binding in the cobalt affinity chromatography column. A control unit will be used to monitor the pH until the solution reaches a desired pH of 7.4. Buffer will be continuously added until the cell suspension achieves a volume of 396 L for $\alpha$ -galactosidase or 374 L $\alpha$ -N-acetylgalactosaminidase. The total volume of the container will be 500 L and at least ten column volumes of equilibration buffer will be added.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	500 L
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$300/bag	

## 15.8. Cobalt Affinity Chromatography Column (P-5, PFD#2)

<u>Description and Function</u>	The cobalt affinity chromatography column will be used to bind the His <sub>6</sub> -tagged $\alpha$ -galactosidase and $\alpha$ -N-acetylgalactosaminidase enzymes and separate them from most of the cell debris, buffers, and B-PER. HisPur Cobalt Superflow Agarose resin (Thermo Fisher Scientific) will be used. Assumes no endotoxin removal.		
<u>Vendor</u>	Pall Corporation		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Cells	10.4	0
	Buffers	1330	146
	Endotoxin	$10.4 \times 10^{-3}$	$10.4 \times 10^{-3}$
	Water	12.1	0
	GalNAc-ase	0.185	0.176
	OR $\alpha$ -galactosidase	0.171	0.162
	Kanamycin	$2.64 \times 10^{-4}$	0
	Chloramphenicol	$1.76 \times 10^{-4}$	0
	Waste	0	1140
<u>Characteristics</u>	Model:	Resolute 280 Chromatography Column	
	Material construction:	Stainless steel, electropolished	
	Required volume:	14.6 L	
	Diameter:	280 mm	
	Height:	237 mm	
	Max linear flowrate:	1200 cm/hr	
	Percent yield:	95%	
	Sterilization:	CIP/SIP	
	Regeneration:	25 times	
	Purity:	$\geq 90\%$	
	Resin:	HisPur Cobalt Superflow Agarose	
	Resin volume:	10.25 L	
	Void fraction:	0.3	
	Binding capacity:	20 g enzyme/L resin	
<u>Operating Conditions</u>	Temperature:	4°C in cold room	
	Pressure:	5.0 atm	
	Linear Flowrate:	150 cm/hr	
<u>Purchase Cost</u>	Column:	\$110,000	

### 15.9. Affinity Chromatography Product Collection Container (P-6, PFD#2)

<u>Description and Function</u>	The product that is eluted from the cobalt affinity chromatography column will be directed to a single-use, disposable 3D bioprocess container. All other column flow-through that came into contact with the cell culture materials will be directed to the waste tank for biowaste heat inactivation and neutralization. Buffers that did not come into contact with biological material will be directed to a waste tank for immediate neutralization.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	200 L
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$250/bag	

## 15.10. Hydrophobic Interaction Chromatography Column (P-7, PFD#2)

<u>Description and Function</u>	The hydrophobic interaction chromatography column will be used to exploit the two-fold difference in hydrophathy between endotoxins and the product enzymes and separate them based on hydrophobicity. It serves as an additional step to eliminate any remaining endotoxins in the product suspension and comply with FDA endotoxin standards. POROS Benzyl HIC Resin, cross-linked poly(styrene-divinylbenzene) POROS base beads with aromatic hydrophobic benzyl ligand, that bind highly hydrophobic particles. It is assumed that 90% of endotoxins will be removed.		
<u>Vendor</u>	Pall Corporation		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Buffers	3678	959
	Endotoxin	10.4×10 <sup>-3</sup>	1.04×10 <sup>-3</sup>
	GalNAc-ase	0.176	0.167
	OR α-galactosidase	0.162	0.154
	Waste	0	2709
<u>Characteristics</u>	Model:	Resolute 800 Chromatography Column	
	Material construction:	Stainless steel, electropolished	
	Required volume:	95.9 L	
	Diameter:	800 mm	
	Height:	191 mm	
	Max linear flow rate:	1200 cm/hr	
	Percent yield:	95%	
	Sterilization:	CIP/SIP	
	Regeneration:	10 times	
	Purity:	≥ 90%	
	Resin:	POROS Benzyl HIC Resin	
	Resin volume:	57.5 L	
	Void fraction:	0.4	
	Binding capacity:	2×10 <sup>6</sup> EU/mL, 90% endotoxin removal	
<u>Operating Conditions</u>	Temperature:	4°C in cold room	
	Pressure:	3.0 atm	
	Linear Flowrate:	150 cm/hr	
<u>Purchase Cost</u>	\$250,000		

### 15.11. HIC Product Collection Containers (P-8, PFD#2)

<u>Description and Function</u>	The product that is eluted from the hydrophobic interaction chromatography column will be directed to two single-use, disposable 3D bioprocess containers. All other column flow-through that came into contact with the cell culture materials will be directed to the waste tank for biowaste heat inactivation and neutralization. Buffers that did not come into contact with biological material will be directed to a waste tank for immediate neutralization.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material Construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	500 L
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$300/bag	

## 15.12. Endotoxin Affinity Chromatography Column (P-9, PFD#2)

<u>Description and Function</u>	The endotoxin affinity chromatography column will be used to bind endotoxins remaining in the product suspension and separate them from the α-galactosidase and α-N-acetylgalactosaminidase products. Pierce High-Capacity Endotoxin Removal Resin (Thermo Fisher Scientific), porous cellulose beads coated with poly(ε-lysine) to act as affinity ligands to endotoxins, will be used. It is assumed that 99% of endotoxins will be removed.		
<u>Vendor</u>	Pall Corporation		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Buffers	1240	19.2
	Endotoxin	1.04×10 <sup>-3</sup>	1.04×10 <sup>-5</sup>
	GalNAc-ase	0.167	0.159
	OR α-galactosidase	0.154	0.146
	Waste	0	1220
<u>Characteristics</u>	Model:	Resolute 280 Chromatography Column	
	Material construction:	Stainless steel, electropolished	
	Required volume:	9.60 L	
	Diameter:	280 mm	
	Height:	156 mm	
	Max linear flow rate:	1200 cm/hr	
	Percent yield:	95%	
	Sterilization:	CIP/SIP	
	Regeneration:	10 times	
	Purity:	≥ 90%	
	Resin:	Pierce High-Capacity Endotoxin Removal Resin	
	Resin volume:	5.75 L	
	Void fraction:	0.4	
	Binding capacity:	2×10 <sup>6</sup> EU/mL, 99% endotoxin removal	
<u>Operating Conditions</u>	Temperature:	4°C in cold room	
	Pressure:	3.0 atm	
	Linear Flowrate:	150 cm/hr	
<u>Purchase Cost</u>	\$110,000		

### 15.13. Endotoxin Affinity Chromatography Product Collection Container (P-8, PFD#2)

<u>Description and Function</u>	The product that is eluted from the endotoxin affinity chromatography column will be directed to a single-use, disposable 2D bioprocess container. All other column flow-through that came into contact with the cell culture materials will be directed to the waste tank for biowaste heat inactivation and neutralization. Buffers that did not come into contact with biological material will be directed to a waste tank for immediate neutralization.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material Construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	50 L
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$37.54/bag	



#### 15.14. Ultrafiltration/Diafiltration (P-11, PFD#2)

Description and Function Upon completion of the purification in the three chromatography steps, the enzyme product will undergo a ultrafiltration-diafiltration (UFDf) step using cassette filters, stabilized with a cassette holder in a Cogent Process Scale Tangential Flow Filtration (TFF) System. The filter will have a 30 kD NMWL to allow for ECO-zymes to stay in the retentate and allowing smaller particles, such as the chromatography buffer components, to filter through. It is assumed that 95% of ECO-zyyme will be recovered in this step, with a five percent loss associated with filtration through the cassette membrane.

Vendor EMD Millipore

Operation Batch

<u>Materials Handled</u>	<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
Buffer	31.0	30.5
GalNAc-ase	0.159	0.151
OR $\alpha$ -galactosidase	0.146	0.139
Endotoxin	$1.04 \times 10^{-5}$	$1.04 \times 10^{-5}$
Waste	0	$4.71 \times 10^{-1}$

<u>Characteristics</u>	Model:	Cogent Process Scale TFF System, Pellicon 3 Ultracel filter cassettes
	TFF System material:	Stainless steel AISI 304
	Membrane Filter Material:	Regenerated cellulose, polyethylene
	Filtration Area:	$1.14 \text{ m}^2$
	Flow Rate:	4 L/min
	Maximum Pressure Drop:	2 atm
	Sterilization:	Disposable cassettes, SIP for stainless steel holder
	Percent Yield:	95%

<u>Operating Conditions</u>	Temperature:	4 °C in cold room
	Pressure:	1 atm

<u>Purchase Cost</u>	Cogent Process Scale TFF System:	\$47,999
	Stainless steel cassette holder:	\$995
	100 pack of Ultracel membranes:	\$479.08/pack

### 15.15. Bulk Filtration (Microfiltration) (P-12, PFD#2)

<u>Description and Function</u>	The final step in purification after ultrafiltration-diafiltration (UFDf) will be a microfiltration to dispose of any remaining endotoxins. Disposable Durapore PVDF filter membranes with a pore size of 0.22 µm will be used. These filters are ideal for sterilization and clarification of protein solutions, allowing ECO-zyme to pass through the filter and be collected, while endotoxin remains bound to the filter membrane. The filters are housed in a disposable Millipak holder unit as well. Each batch will pass through the unit twice, removing 99% of endotoxins each time.		
<u>Vendor</u>	EMD Millipore		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	Buffer	30.5	27.5
	GalNAc-ase	0.151	0.136
	OR α-galactosidase	0.139	0.125
	Endotoxin	$1.04 \times 10^{-5}$	$1.04 \times 10^{-9}$
	Waste	0	3.31
<u>Characteristics</u>	Model:	Millipak® Disposable Units, 0.22µm	
	Membrane filter material:	Durapore PVDF	
	Filtration area:	100 cm <sup>2</sup>	
	Maximum pressure drop:	4.1 atm	
	Sterilization:	Disposable	
	Percent yield:	95%	
	Flow rate:	16.5 L/min	
	Protein binding:	150 µg/cm <sup>2</sup>	
	Wettability:	Hydrophobic	
<u>Operating Conditions</u>	Temperature:	4 °C in cold room	
	Pressure:	1 atm	
<u>Purchase Cost</u>	Pack of 3 disposable membranes and holders:	\$503/pack	

### 15.16 Product Storage Tank (P-13, PFD#2)

<u>Description and Function</u>	A final 500 L 3D disposable biocontainer will be used to collect the product solution from bulk microfiltration. The product will be resuspended in 205 or 189 L of water for $\alpha$ -N-acetylgalactosaminidase and $\alpha$ -galactosidase, respectively. The resuspension solution will have a concentration of 200 mM glycine to prevent protein aggregation.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material Construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	500 L
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1 atm
<u>Purchase Cost</u>	\$300/bag	

#### 15.4 Hyperbaric Chamber (not shown, PFD#3)

<u>Description and Function</u>	Donor packed RBCs that are designated for treatment will be emptied in bulk into a storage vessel using a hyperbaric chamber to induce a pressure differential between 0.3 and 0.5 atm between the blood bags and the collection vessel. This will generate pressure-driven flow from the blood bags into the disposable storage container. This unit operation will minimize both the labor force required to empty the number of blood bags required in each batch and the risk of human error and contamination during emptying. Additionally, it allows blood to be emptied quickly and minimize the amount of time it could be exposed to room temperature.	
<u>Vendor</u>	To be custom made by third party manufacturer after discussions with Cummings Properties.	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material construction:	Stainless steel AISI 304, PVC tubing for blood bags
	Sterilization:	Disposable dry blood bags and tubing, CIP/SIP when needed
	Pressure differential:	0.5-1.0 atm
	Number of bags to empty:	1973 bags max.
	Blood bags emptying rate	264 bags/run
	Run time:	2.5 hours/batch
<u>Operating Conditions</u>	Temperature:	4°C
	Pressure:	0.5-1.5 atm
<u>Purchase Cost</u>	\$100,000	

### 15.5 Blood Collection Container (not shown, PFD#3)

<u>Description and Function</u>	The blood emptied from the blood bags by pressure-driven flow will be collected in a 500 L 3D bioprocess container. The container will be kept at 4°C at all times to preserve the biological activity of the RBCs.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material Construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	500 L
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1 atm
<u>Purchase Cost</u>	\$300/bag	

#### 15.4 Disk Stack Centrifuge (P-1, PFD#3)

<u>Description and Function</u>	The centrifuge will be used to separate the RBCs (solids) from the spent additive preservation solution (liquid). The output will be 60% by mass water and 40% by mass RBCs.		
<u>Vendor</u>	Alfa Laval		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	<b>For type-A</b>		
	RBC suspension	0.595	0.559
	<b>For type-B</b>		
	RBC suspension	0.586	0.551
<u>Characteristics</u>	Model:	Culturefuge 100	
	Centrifuge type:	Disk stack	
	Material construction:	Mo-bearing austenitic steel for body and cover; cast grey iron bottom frame	
	Finish:	Electro-polished	
	Hydraulic capacity:	2,000 L/hr capacity	
	G-force:	12,220 g maximum	
	Bowl speed:	9550 rpm maximum	
	Percent Yield:	95%	
	Sterilization	CIP/SIP	
<u>Operating Conditions</u>	Temperature:	4°C	
	Pressure:	1 atm	
	Flowrate:	200 L/hr	
<u>Purchase Cost</u>	\$46,500/unit		

## 15.5 WAVE Bioreactors (P-2, PFD#3)

<u>Description and Function</u>	The conversion process in which type-A and type-B RBCs will be converted to type-O cells via enzymatic conversion will take place in multiple WAVE bioreactors. The total working volume of approximately 413 L will be divided among five WAVE bioreactors that have a maximum working volume of 100 L. The reduced volume in each reactor will also allow for better mixing of the RBCs and conversion enzymes, hopefully increasing conversion efficacy.		
<u>Vendor</u>	Sartorius Stedim Biotechnology Group		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	<b>For type-A</b>		
	RBC suspension	0.559	0.526
	GalNAc-ase	0.118	0.111
	<b>For type-B</b>		
	RBC suspension	0.551	0.518
	$\alpha$ -galactosidase	$3.95 \times 10^{-3}$	$3.71 \times 10^{-3}$
<u>Characteristics</u>	Model:	BIOSTAT <sup>®</sup> RM bioreactor system linked to BIOSTAT <sup>®</sup> control tower, FLEXSAFE <sup>®</sup> RM disposable bag	
(for each reactor)	Material construction:	Stainless steel AISI 304 control tower, stainless steel ABS rocker, low-density polyethylene bags.	
	Dimensions:	740mm x 720mm	
	Heating method:	Open thermostat system with circulation pump and automatic cooling water valve	
	Heating capacity:	$2 \times 600$ W	
	Percent Yield:	95%	
	Sterilization:	Disposable, single-use bags: CIP/SIP for rocking base	
	Rocking speed:	15 rocks/min	
	Rocking angle:	10°	
	Maximum working volume:	100 L	
	Total volume:	200 L	

<u>Operating Conditions</u>	Temperature:	25°C
	Pressure:	1.0 atm
	pH:	7.4-7.6
	pO <sub>2</sub> :	40%
	Reaction time:	1 hour

<u>Purchase Cost</u>	\$20,000/unit
	\$200/bag



#### 15.4 Disk Stack Centrifuge (P-3, PFD#3)

Description and Function The centrifuge will be used to separate the RBCs (solids) from the spent enzymatic solution (liquid). The output will be 60% by mass water and 40% by mass RBCs. This step is taken as a first purification step to separate enzymes from converted RBCs, given that remaining enzymes in solution have found to cause toxicity in pre-clinical and early clinical studies.

Vendor Alfa Laval

Operation Batch

<u>Materials Handled</u>	<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
<b>For type-A</b>		
RBC suspension	0.525	0.494
GalNAc-ase	0.111	0.104
<b>For type-B</b>		
RBC suspension	0.518	0.487
$\alpha$ -galactosidase	$3.71 \times 10^{-3}$	$3.49 \times 10^{-3}$

Characteristics

Model:	Culturefuge 100
Centrifuge type:	Disk stack
Material construction:	Mo-bearing austenitic steel for body and cover; cast grey iron bottom frame
Finish:	Electro-polished
Hydraulic capacity:	2,000 L/hr capacity
G-force:	12,220 g maximum
Bowl speed:	9550 rpm maximum
Percent Yield:	95%
Sterilization	CIP/SIP

Operating Conditions

Temperature:	4°C
Pressure:	1 atm
Flowrate:	200 L/hr

Purchase Cost \$46,500/unit

### 15.11 Diafiltration (P-4, PFD#3)

Description and Function To remove any remaining enzyme from the converted RBCs to prevent immunogenic responses in patients, diafiltration will be performed using a Cogent Process-Scale Tangential Flow Filtration System with 500 kD nominal molecular weight limit (NMWL) microfiltration filters. The NMWL will allow RBCs to remain in the retentate and return to the storage tank while enzymes are continuously removed from the system. Saline will continuously be added to the process to ensure the suspension buffer of the RBCs has an osmolality equivalent to biological conditions to prevent hemolysis. It is assumed that 95% the treated RBCs will be recovered in this step, with a 5% loss associated with filtration through the cassette membrane or lysis. We assume near complete removal of enzyme to the point that remaining presence is negligible.

Vendor EMD Millipore

Operation Batch

<u>Materials Handled</u>	Input (kg/batch)	Output (kg/batch)
<b>For type-A</b>		
RBC suspension	0.494	0.465
GalNAc-ase	0.104	0
Waste	0	0.133
<b>For type-B</b>		
RBC suspension	0.503	0.458
$\alpha$ -galactosidase	$3.49 \times 10^{-3}$	0
Waste	0	$4.85 \times 10^{-2}$

<u>Characteristics</u>	Model:	Cogent Process Scale TFF System, Pellicon 3 Ultracel filter cassettes
	TFF system material:	Stainless steel AISI 304
	Filter Material:	Regenerated cellulose, polyethylene
	Filtration Area:	1.14m <sup>2</sup>
	Flow Rate:	4 L/min
	Maximum Pressure Drop:	2 atm
	Sterilization:	Disposable cassettes, SIP for stainless steel holder
	Percent Yield:	95%

<u>Operating Conditions</u>	Temperature:	4 °C in cold room
	Pressure:	1 atm

Purchase Cost

Cogent Process Scale TFF System:	\$47,999
Stainless steel cassette holder:	\$995
100 pack of Ultracel membranes:	\$479.08/pack

## 15.9 Cryogel-based Affinity Chromatography Column (P-6, PFD#3)

Description and Function The cryogel-based affinity chromatography column will be used to bind non-converted RBCs to separate them from successfully converted RBCs. This is done as a precautionary measure to ensure that the finalized product does not have antigen- incompatible RBCs that may cause a harmful immunogenic response in patients. Instead of traditional resin, the column will be packed with a polymer-based cryogel that is coated in a protein A-IgG antibody coat that bind the antigens of either type-A or type-B RBCs. It is assumed that there is a 95% recovery of converted RBCs.

Vendor Pall Corporation

Operation Batch

<u>Materials Handled</u>	<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
<b>For type-A</b>		
RBC suspension	0.465	0.437
Buffers	2125	702
Waste	0	1423
<b>For type-B</b>		
RBC suspension	0.458	0.430
Buffers	2125	702
Waste	0	1423

Characteristics

Model:	Resolute 280 Chromatography Column
Material construction:	Stainless steel, electropolished
Required volume:	18.0 L
Diameter:	280 mm
Height:	292 mm
Max linear flow rate:	1200 cm/hr
Percent yield:	95%
Sterilization:	CIP/SIP
Regeneration:	5 times
Purity:	≥ 90%
Gel volume:	14.42 L
Affinity IgG:	Mouse monoclonals: anti-blood Group A antigen Ab [clone 9A] & anti-blood Group B antigen Ab [HEB-29]
Void fraction:	0.4

<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	5.0 atm
	Linear Flowrate:	100 cm/hr

<u>Purchase Cost</u>	\$110,000
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### 15.10 Cryogel-based Affinity Chromatography Product Collection Container (P-7, PFD#3)

<u>Description and Function</u>	The flow-through of the cryogel-based affinity chromatography column, which contains successfully converted RBCs, will be collected in four single-use, disposable 2D bioprocess container in equal volumetric amounts. All other column flow-through that came into contact with the biological substances will be directed to the waste tank for biowaste heat inactivation and neutralization. Unconverted RBCs bound to the column will be released via mechanical stress and also directed to the aforementioned waste tank. Buffers that did not come into contact with biological material will be directed to a waste tank for immediate neutralization.	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material Construction:	Sterile single-use CX5-14 multi-layer plastic
	Volume:	200 L/bag
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$250/bag	

### 15.11 Diafiltration (P-8, PFD#3)

Description and Function To remove any remaining enzyme from the converted RBCs to prevent immunogenic responses in patients, diafiltration will be performed using a Cogent Process-Scale Tangential Flow Filtration System with 500 kD nominal molecular weight limit (NMWL) microfiltration filters. The NMWL will allow RBCs to remain in the retentate and return to the storage tank while enzymes are continuously removed from the system. Saline will continuously be added to the process to ensure the suspension buffer of the RBCs has an osmolality equivalent to biological conditions to prevent hemolysis. It is assumed that 95% the treated RBCs will be recovered in this step, with a 5% loss associated with filtration through the cassette membrane or lysis. Assuming complete removal of all buffer salts and components.

Vendor EMD Millipore

Operation Batch

<u>Materials Handled</u>	<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
<b>For type-A</b>		
RBC suspension	0.437	0.410
Buffer salts	702	0
Waste	0	1423
<b>For type-B</b>		
RBC suspension	0.430	0.404
Buffer salts	702	702
Waste	0	1423

Characteristics

Model:	Pellicon 3 cassettes
Membrane Filter Material:	Regenerated cellulose, polyethylene
Filtration Area:	1.14m <sup>2</sup>
Flow Rate:	4 L/min
Maximum Pressure Drop:	2 atm
Sterilization:	Disposable cassettes, SIP for stainless steel holder
Percent Yield:	95%

Operating Conditions

Temperature:	4 °C in cold room
Pressure:	1 atm

Purchase Cost

Cogent Process Scale TFF System:	\$47,999
Stainless steel cassette holder:	\$995
100 pack of Ultracel membranes:	\$479.08/pack

#### 15.4 Disk Stack Centrifuge (P-9, PFD#3)

<u>Description and Function</u>	The centrifuge will be used to separate the RBCs (solids) from the spent enzymatic solution (liquid). The output will be 60% by mass water and 40% by mass RBCs. This step is taken to separate RBCs from any remaining buffer solution. It also allows greater control of the final volume in which the RBCs are suspended to facilitate final packaging.		
<u>Vendor</u>	Alfa Laval		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/batch)</b>	<b>Output (kg/batch)</b>
	<b>For type-A</b>		
	RBC suspension	0.410	0.386
	<b>For type-B</b>		
	RBC suspension	0.404	0.381
<u>Characteristics</u>	Model:	Culturefuge 100	
	Centrifuge type:	Disk stack	
	Material construction:	Mo-bearing austenitic steel for body and cover; cast grey iron bottom frame	
	Finish:	Electro-polished	
	Hydraulic capacity:	2,000 L/hr capacity	
	G-force:	12,220 g maximum	
	Bowl speed:	9550 rpm maximum	
	Percent Yield:	95%	
	Sterilization	CIP/SIP	
<u>Operating Conditions</u>	Temperature:	4°C	
	Pressure:	1 atm	
	Flowrate:	200 L/hr	
<u>Purchase Cost</u>	\$46,500/unit		



### 15.13 Product Storage Containers (P-10, PFD#3)

<u>Description and Function</u>	The centrifuged RBC mass will be directed and resuspended in Adsol-1 additive solution (previously prepared in a mixing tank) in four single-use, disposable 2D bioprocess container in equal volumetric amounts. The stored converted RBCs will be kept in a cold room on a rocker at gentle agitation to prevent settling until they can be packaged	
<u>Vendor</u>	Thermo Fisher Scientific	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material Construction:	Sterile plastic
	Volume:	200 L/bag
	Sterilization:	Disposable
<u>Operating Conditions</u>	Temperature:	4°C in cold room
	Pressure:	1 atm
<u>Purchase Cost</u>	\$250/bag	

### 15.15 Small Peristaltic Pumps (All main pumps in PFDs)

<u>Description and Function</u>	Peristaltic pumps are used to transfer unit contents throughout the process. All peristaltic pumps are specifically designated for use in the <i>E. coli</i> upstream and downstream processes to the high viscosity of the cell suspensions. The consistent flow ensures accuracy in dispensing flow from unit to unit. A brushless motor allows for speed control of the pump.	
<u>Vendor</u>	Cole Parmer	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	Masterflex Model 77924-80
	Material construction:	Stainless Steel AISI 304
	Flow rate range:	0.001-3,400 mL/min
	Maximum Pressure Drop:	2.07 atm
	Power requirement:	253 W
<u>Operating Conditions</u>	Temperature:	4-37°C
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$3,635/pump	

### 15.15 Large Peristaltic Pumps (Used for regeneration buffers)

<u>Description and Function</u>	Peristaltic pumps are used to transfer unit contents throughout the process. All peristaltic pumps are specifically designated for use in the <i>E. coli</i> upstream and downstream processes to the high viscosity of the cell suspensions. The consistent flow ensures accuracy in dispensing flow from unit to unit. A brushless motor allows for speed control of the pump.	
<u>Vendor</u>	Watson Marlow	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	840 Series Hygienic Pump
	Tubing:	Gore® STA-PURE® PFL
	Material construction:	Stainless Steel 316
	Flow rate range:	120-8,000 L/hr
	Maximum Pressure Drop:	3.5 atm
	Power requirement:	0.2 W
<u>Operating Conditions</u>	Temperature:	4-37°C
	Pressure:	1.0 atm
<u>Purchase Cost</u>	\$25,000/pump	

### 15.15 Quattroflow Quaternary Diaphragm Pumps (All pumps shown in PFD#3)

<u>Description and Function</u>	The Quattroflow quaternary diaphragm pumps mimic cardiovascular pumping in the human body to prevent shear-induced hemolysis. All Quattroflow pumps are specifically designated for use in the RBC conversion treatment process. Each stroke of the 4-piston diaphragm is generated by an eccentric shaft connected to an electric motor. Each Quattroflow pump comes with an integrated PID controller for optimal flow rate adjustments.	
<u>Vendor</u>	PSG Dover	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	QF1200S-CV
	Material construction:	Stainless Steel AISI 304
	Flow rate range:	10-1,200 L/hr
	Maximum Pressure Drop:	6 atm
	Power requirement:	0.75 W
<u>Operating Conditions</u>	Temperature:	4°C
	Pressure:	1.0 atm
	Flow rate:	350 L/hr
<u>Purchase Cost</u>	\$10,772/pump	

**Section 16:**  
**Additional Equipment**  
**Description**

## 16. Additional Equipment Description

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There are a number of units that will be needed for the completion of this project but are not shown in the process flow diagrams. These pieces of equipment are required for sterile cell culture and storage, sterile buffer preparation, proper waste disposal, and product verification steps, thus allowing for good manufacturing practices.

### 16.1. Biosafety Cabinet

Two class II biosafety cabinets will be required for sterile cell culture spaces. Each biosafety cabinet will come equipped with mounted motor driven blowers to draw directional mass airflow around a user and into the air grille to protect the operator. The airflow drawn into the air grille will then be HEPA-filtered and exhausted into the environment. Additionally, a column of HEPA filtered, sterile air is blown downward at the front of cabinet, over products and processes to prevent contamination. The first biosafety cabinet will be designated to handle *E. coli* BL21(DE3) cells exclusively and is provide a sterile environment to transfer the contents of thawed vials of cells for inoculating the ambr<sup>®</sup> 250 bioreactor. The second biosafety cabinet will be designated to handle any RBCs or other blood-derived materials/products for research purposes. The rented laboratory space will provide both biosafety cabinets, which is factored in the rental costs in annual expenses.

### 16.2. Cell Bank

Engineered *E. coli* BL21(DE3) will kept frozen in a -80°C freezer in 1.5-mL vials at a concentration of  $7.95 \times 10^6$  cells/mL. 250 vials annually to allow for a year's worth of production of  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase, with additional an additional stock in the event of complications with the cell line. The cell solution will also be 20% glycerol by volume. The addition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. All vials will be prepared in a class II biosafety cabinet.

### 16.3. Refrigeration

A -80°C freezer will be necessary to maintain the frozen cell stock and certain reagents for long-term storage. A -20°C freezer will also be purchased to maintain certain reagents at their

proper storage temperature. Both the ultra-low temperature freezer and the -20°C freezers will be purchased from Thermo Fisher Scientific for \$30,000 and \$8,700, respectively.

#### **16.4. Biowaste and Neutralization Tanks**

All waste streams that have been in contact with cell culture or debris will be directed to 10,000 L stainless steel tank, which will be purchased from Sharpsville Container for \$2,500. The tank's contents will undergo proper heat inactivation before disposal to ensure that there are no remaining proliferating *E. coli* cells that could cause environmental health hazards. This tank will be jacketed to be able to reach and sustain a temperature of 121°C for approximately 11 minutes via a digital PID controller unit to complete a 25-log reduction of bacteria.

Cell-free waste streams will be directed to an additional 10,000 L stainless steel tank, also purchased from Sharpsville Container. This tank will mainly collect the different buffers used in the chromatography steps, which will undergo neutralization to adjust the waste to a pH of 7.0. After neutralization, this waste will be discarded by the laboratory facility into the sewer line.

The disposal of non-liquid waste, which primarily includes all single-use, disposable bags, containers, and reactor vessels, will be contracted to the biohazardous waste disposal unit of Stericycle. The costs of outsourcing this task will be added to the annual expenses.

#### **16.5. Water-for-Injection Generator**

A multiple effect still will be used to purify water for use in preparing media, preparing all buffers and additive solutions, and conducting SIP procedures. FDA standards require that pharmaceutical and biological products injected or transfused into patients do not touch any water other than water-for-injection (WFI) to ensure sterility and reduce contamination risks. The WFI will also ensure sterility of the process equipment and eliminate cross-contamination issues. The PyroPure Multiple Effect Still will be purchased from the Paul Mueller Company for \$20,000.

#### **16.6. Water Treatment Package**

Although WFI will be used for the water requirements in the process, U.S. Pharmacopeia (USP) grade water is needed for temperature control needs in the bioreactor processes. This water will not be in direct contact with the cell culture suspension. It will be inserted into the reactors'

for heating and cooling procedures. The cost for the water treatment package is included in the operations costs of the annual expenses.

#### **16.7. Clean Steam Generator**

Clean steam will be necessary to conduct SIP procedures for numerous process units. The generator for will produce clean steam from WFI, supplied by the WFI generator. The clean steam generator will be purchased from BMT USA for \$60,000.

#### **16.8. Buffer Transfer Bags**

Disposable bioprocess containers (Thermo Fisher Scientific) will be used to prepare the equilibration, wash, elution, and regeneration buffers for all chromatography columns. Each column will require five containers (with its respective holder): one for equilibration, wash, elution, regeneration, and CIP reagents.

#### **16.9. Filter Integrity**

Although disposable filters are used throughout the process, they must be tested before each use to ensure they are not clogged, torn, or generally unusable. The filter integrity test will help minimize losses due to filter manufacturing error. The test will be purchased from and performed by EMD Millipore for \$3,500 upon ordering the filters.

#### **16.10. Purified Air Generator**

A purified, zero air generator for all space in the laboratory facilities will be purchased from Domnick Hunter for \$2,500 per unit. This device will produce a continuous stream of organic impurity free air from an external dry compressed air source. The system will provide clean air to bioreactors and fermenters, clean rooms, and process equipment.

#### **16.11. Laboratory Information Management System**

Laboratory information management system (LIMS) will be implemented for workflow, data tracking support, and data exchange interfaces. It will be used to enforce standard operating procedures (SOPs) and to provide defined workflows to analysts. Its functions will include, but are not limited to: sample management (login, registration integrity, life cycle tracking), chain of custody, instrument integration for data collection automation, automatic results reduction &



flagging, integration with third party systems (ERP/HR/CDS), workflow management, work allocation & scheduling to reduce as of yet unidentified bottlenecks, electronic document management, lot & batch management, instrument management (maintenance, scheduling), data security & integrity, multivariate data analysis, and quality assurance and control.

#### **16.12. Portable Pump on Cart**

In the event of pump malfunction in either the *E. coli* upstream/downstream processes or the RBC treatment, two extra peristaltic pumps and two extra Quattroflow quaternary diaphragm pumps will be stored as replacements. These pumps will be mounted onto carts for easy intra-facility movement in the event of a pump's mechanical malfunction.

#### **16.13. Quality Control Lab Equipment**

Quality control analysis will be divided between the enzyme production and RBC conversion steps. These product verification steps must be passed for conversion enzymes to be used to treat RBCs and for successfully converted RBCs to proceed to final packaging. Less than 10 µg of each enzyme per batch will be required for quality control analysis. 500 mL of both converted and non-converted blood will be aliquoted and kept for flow cytometry and thin layer chromatography (TLC) analysis to ensure that the final product contains minimal traces of non-converted RBCs.

##### **16.13.1. Protein Gel Materials**

Protein gels will be run for each to confirm the identity of the isolated enzymes from the *E. coli* culture. Additionally, the protein gels will help identify possible protein variations that could be associated with genetic drift or mutations in the *E. coli* cell line. Thus, this control step has a secondary purpose of validating cell productivity. The materials needed include hydrochloric acid, 30% acrylamide solution, SDS ammonium persulfate, TEMED, glycine, EDTA, 50% glycerol solution, mercaptoethanol, bromophenol blue, and pre-stained molecular weight markers. These materials will be purchased from BioRad for \$1,540 per batch.

##### **16.13.2. NanoDrop**

The NanoDrop 2000 UV-Vis Spectrophotometer will be used to determine the concentration of  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase in their respective final

solutions. Once known, the concentration will be used to determine the appropriate volumes of enzyme to use for treating RBCs. This unit will be purchased from Thermo Fisher Scientific for \$9,100.

#### **16.13.3. Endotoxin Test Kit**

Endotoxin detection will be done using a Kinetic Turbidimetric Limulus Amebocyte Lysate (LAL) test. A test kit will be purchased annually to ensure that all batches of conversion enzyme have an endotoxin level below the FDA standard of 0.1 EU/mL. If a batch does not pass this test, it will be discarded. The kit will be purchased from Lonza at \$1,000 per kit. Each kit can be used 192 times.

#### **16.13.4. Flow Cytometer**

Flow cytometry analysis will be performed on native and enzymatically converted RBCs using a BD LSR II flow cytometer (BD Biosciences) with mouse monoclonal antibody reagents specific to human A or B antigens on the RBC surface. The flow cytometer will come equipped with 488, 594, and 633 nm lasers. The final RBC population must have less than 1.0% non-converted RBCs to be considered acceptable for transfusion.

The principle of action is that if any remaining antigens are not cleaved on the red blood cell surface, then fluorophore-labeled primary antibodies specific to the antigens can be used to bind to them. The fluorophores will be Alexa Fluor 546 or 547 secondary antibodies and will bind specifically to the primary antibodies. The flow cytometer will perform a high-throughput analysis of the liquid blood sample, one cell at a time, by shining lasers of specific wavelengths on them. Successfully converted cells will emit no signal, while cells that still contain antigens will give off signal when they are excited by 594 or 633 nm wavelength lasers.

#### **16.13.5. TLC for Glycolipid Analysis**

The TLC of native and converted RBC glycolipids should show that converted cells no longer have glycolipid bands associated with the sugars that constitute the A and B antigens. RBC membranes will be extracted by homogenization in ten volumes of isopropanol/hexane/water (55:25:20 vol/vol/vol), filtered, evaporated and partitioned by Folch extraction method. The upper phase will be collected, evaporated, dialyzed and applied to a DEAE Sephadex column. The pass-

through fraction will contain the total upper neutral lipids, and the monosialyl fraction will be eluted with 0.05 M ammonium acetate, evaporated and dialyzed against water to remove acetate. Total upper neutral and monosialyl glycolipid fractions will be analyzed by high-performance thin-layer chromatography. TLC plates (Merck) will be developed using the solvent system chloroform/methanol/0.5 M CaCl<sub>2</sub> in water (50:40:10, vol/vol/vol). Glycolipids will be stained by heating with 0.05% orcinol in 0.5 M H<sub>2</sub>SO<sub>4</sub>.

# **Section 17:**

## **Equipment Cost Summary**

## 17. Equipment Cost Summary

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### 17.1. Major Unit Operation Costs

The total purchase cost for the major operation equipment is \$1,948,914. Using a bare module factor of 3.21, the bare module cost is approximately \$6.23 million. This bare module factor is determined assuming that the cost of installation materials is 71% of equipment purchase cost; the cost of installation labor is 54%; the cost for freight, insurances, and taxes is 9%; the cost of construction overhead is 57%; and the cost of contractor engineering expenses is 30%. A bare module factor of 3.21 yields a highly conservative estimate of the bare module cost for a process that relies heavily on off the shelf equipment as this process does. In reality, however, the cost of installation materials and contractor engineering expenses will be significantly less since most items are purchased already manufactured. The real bare module cost for this process is likely lower than the estimated \$6,231,443. However, for the purpose of the profitability analysis the upper bound is used to provide a stricter analysis on the feasibility of this project. The breakdown of the major unit operations costs, including manufacturer, number of units required, purchase cost, and final bare module cost, is shown in Table 17.1.1.

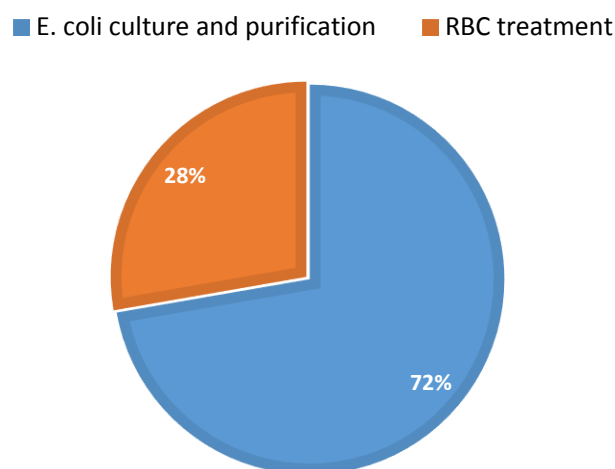
The most expensive pieces of equipment are the Resolute chromatography chromatography columns, followed by the production bioreactor and the WAVE bioreactor setup. It should be noted that although the Resolute 280 chromatography column is used for the cryogel-based affinity chromatography column, similar to the cobalt ion and endotoxin affinity chromatography steps, an additional cost of \$90,000 is added to the original purchase cost of \$110,000 for customization to add a piston to apply mechanical stress for releasing bound cells.

The costs of genetically engineering the *E. coli* cell lines was included in the major unit operation costing given their similarity to purchasing equipment: it is a one-time investment. A bare module factor of 1.0 was applied for the costing of the gene editing since it does not require any of the previously aforementioned costs.

It should be noted that the major unit operation costs include two Cogent M1 Tangential Flow Filtration systems that are not previously discussed in the process description. These two units are being purchased as backups in the event that the Cogent Process Scale Tangential Flow Filtration System suffers from a malfunction and cannot be used. The elimination of enzymes and

buffers in these products are important for marketability purposes. If they cannot be eliminated, the RBCs will have enzymatic content that could cause an immunogenic response in patients, defeating the purpose of this project. Thus, for equipment functionality concerns, these units were included in our calculations. The presence of these two units will also allow for more increasing processing capability in the event that expansion occurs or if donated whole blood, which requires a separate sets of ultrafiltration and diafiltration processes, becomes incorporated into the plant design.

The unit operation costs associated with the *E. coli* process are significantly higher than the treatment of RBCs (approximately 2.6-fold higher), as shown in Figure 17.1.1.



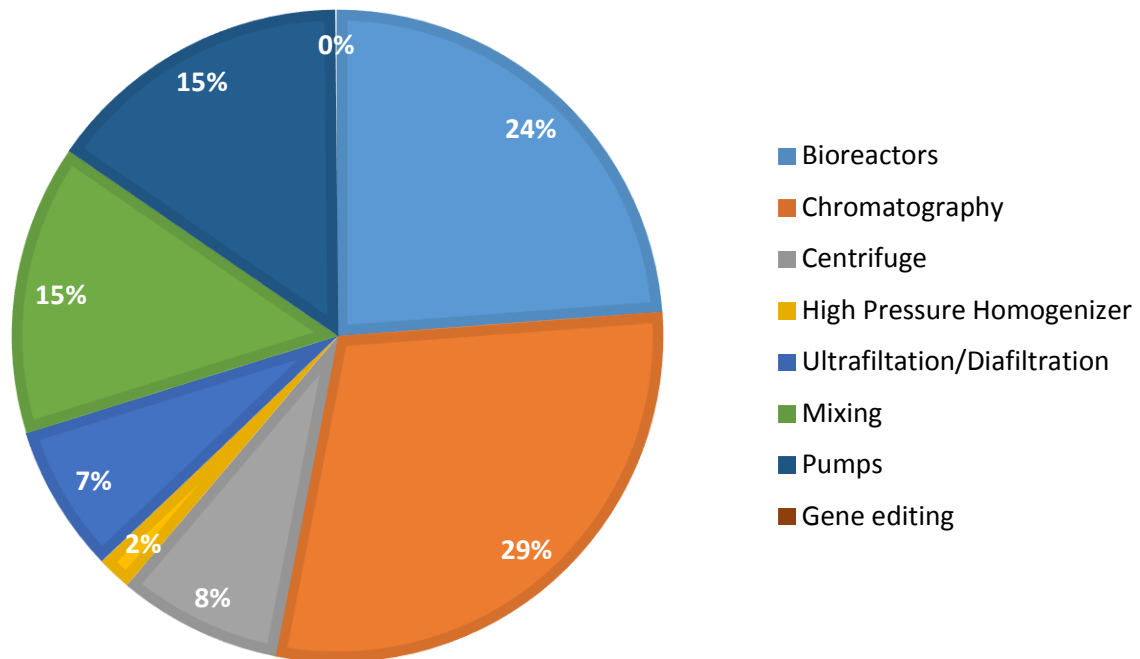
**Figure 17.1.1.** Contributions of both the *E. coli* culture/purification and the conversion treatment of RBCs to the total bare module costs of the major unit operation costs. Given the complexity of ensuring that endotoxins concentrations are lowered to a concentration considered acceptable by the FDA, the multiple incorporated chromatography column steps imposes significant costs on just the microbial fermentation.

The total purchase cost for the additional equipment is \$1.17 million. The costing of other common units required in the processes is described in Table 17.1.2. Using a bare module factor of 3.21, the bare module cost is \$3.74 million. Of these units, only the cold rooms and hyperbaric chamber will need to be designed and fabricated, which is accounted for in their total bare module

costs. Other equipment, such as pumps, freezers, and refrigerators, will be purchased as off the shelf units, which will likely result in a bare module cost lower than the predicted value. However, we again choose to analyze the economics from this standard of 3.21 to provide a clear upper bound on what equipment purchases costs will be for this project.

The most significant costs are associated with the construction of the several cold rooms required throughout the process (\$1.7 million). The price for construction of the cold room was quoted at \$170 per square foot (ANC Cold Storage Construction, Inc.). Each cold room was designed to be 900 ft<sup>2</sup> in area, but this number will be subject to change after further consultation with Cummings Properties and process equipment vendors. For costing assessment, however, we believe that this area is sufficiently large for each to house RBC transfusion units, downstream purification equipment for the microbial culture, or the RBC treatment process equipment. This will thus give us an upper bound on the cost estimation of the cold rooms and allow for more stringent analysis of this process design.

The total breakdown of all equipment costs is shown in Figure 17.1.2. The total bare module cost of all units is \$9,976,534. Thus, it is estimated that just on equipment purchases alone, an initial capital investment of \$10 million would be required to just have the machinery with which the bacterial growth and RBC treatment processes can be performed. For plant operating, a complete, initial capital investment of \$25.6 million will be required. This means that equipment costs account for roughly 37.5% of the initial investment.



**Figure 17.1.2.** Complete breakdown of all equipment that need to be purchased for plant operation. The total bare module cost is \$9.98 million. We expect this number to be lower given that the bare module factor of 3.21 applied to nearly all units in this design was deemed too high by numerous industrial consultations. Since these units are purchased off-shelf, there are no major installation material costs will be lower.



**Table 17.1.1.** Major equipment purchases costs, including information on manufacturers, number of units, and the final, expected bare module cost associated with each unit.

Equipment Description	Vendor	Type	Units	Purchase Cost (per unit)	Total Purchase Cost	Total Bare Module Cost
Innoculum Bioreactor	Sartorius	Process Machinery	1	\$ 45,799	\$ 45,799	\$ 147,015
Seed Bioreactor	Eppendorf	Process Machinery	1	\$ 100,000	\$ 100,000	\$ 321,000
Bioreactor	Thermofisher	Process Machinery	2	\$ 150,000	\$ 300,000	\$ 963,000
Cobalt Affinity Chromatography	Pall	Process Machinery	1	\$ 110,000	\$ 110,000	\$ 353,100
Endotoxin Affinity Chromatography	Pall	Process Machinery	1	\$ 110,000	\$ 110,000	\$ 353,100
Hydrophobic Interaction Chromatography	Pall	Process Machinery	1	\$ 250,000	\$ 250,000	\$ 802,500
Disk Stack Centrifuge	Alfa-Laval	Process Machinery	4	\$ 46,500	\$ 186,000	\$ 597,060
High Pressure Homogenizer	Microfluidics	Process Machinery	1	\$ 38,740	\$ 38,740	\$ 124,355
WAVE Bioreactor	Sartorius	Process Machinery	5	\$ 20,000	\$ 100,000	\$ 321,000
Cryogel Affinity Chromatography	Pall	Process Machinery	1	\$ 200,000	\$ 200,000	\$ 642,000
Cogent Process Scale TFF System	EMD Millipore	Process Machinery	3	\$ 47,999	\$ 143,997	\$ 462,230
Cogent M1 TFF	EMD Millipore	Process Machinery	2	\$ 12,000	\$ 24,000	\$ 77,040
HyPerforma Single-Use Mixer	Sartorius	Process Machinery	2	\$ 165,000	\$ 330,000	\$ 1,059,300
<i>E. coli</i> cell line engineering	GenScript	Biological Processing	2	\$ 5,400	\$ 10,800	\$ 10,800
Gene fabrication	GenScript	Biological Processing	2	\$ 159	\$ 318	\$ 318
Total					\$ 1,949,654	\$ 6,233,819

**Table 17.1.2.** Additional, common equipment purchase costs, including information on manufacturers, number of units, and the final, expected bare module cost associated with each unit.

Equipment Description	Vendor	Type	Units	Purchase Cost (per unit)	Total Purchase Cost	Total Bare Module Cost
Masterflex 77924-80 L/S	Cole-Palmer	Process Machinery	39	\$ 3,635	\$ 141,765	\$ 455,066
840 Series Hygienic Pump	Watson Marlow	Process Machinery	4	\$ 25,000	\$ 100,000	\$ 321,000
Quattroflow Quaternary Diaphragm Pump	PSG Dover	Process Machinery	10	\$ 10,772	\$ 107,720	\$ 345,781
Spark Multimode Microplate Reader	Tecan	Process Machinery	1	\$ 2,450	\$ 2,450	\$ 7,865
Nanodrop VIS Spectrophotometer	Thermo Scientific	Process Machinery	1	\$ 9,140	\$ 9,140	\$ 29,339
BD Biosciences LSRII Flow Cytometer	Biosciences	Process Machinery	1	\$ 55,115	\$ 55,115	\$ 176,919
4°C Refrigerator	Thermo Scientific	Storage	1	\$ 13,600	\$ 13,600	\$ 43,656
Freezer (-20°C)	Thermo Scientific	Storage	4	\$ 8,700	\$ 34,800	\$ 111,708
Freezer (-80°C)	Thermo Scientific	Storage	1	\$ 30,000	\$ 30,000	\$ 96,300
Clean Steam Generator	BMT USA	Process Machinery	1	\$ 60,000	\$ 60,000	\$ 192,600
Clean Air Generator	Domnick Hunter	Process Machinery	1	\$ 2,500	\$ 2,500	\$ 8,025
Water for Injection Supplier	Paul Mueller Co.	Process Machinery	1	\$ 20,000	\$ 20,000	\$ 64,200
Digital PID controller units	Omega	Computers, Software, Etc.	30	\$ 300	\$ 9,000	\$ 28,890
Control software	Omega	Computers, Software, Etc.	1	\$ 20,865	\$ 20,865	\$ 66,977
Cold room	N/A	Fabricated Equipment	1	\$ 459,000	\$ 459,000	\$ 1,473,390
Hyberbaric chamber	N/A	Fabricated Equipment	1	\$ 100,000	\$ 100,000	\$ 321,000
Total					\$ 1,165,955	\$ 3,742,716

# **Section 18:**

## **Fixed Capital Investment Summary**

## 18. Fixed Capital Investment Summary

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A breakdown of the required investments costs, beginning with total bare module costs and ending with the required total capital investment for the project is shown in Table 18.2. \$14 million will be requested to cover the minimum necessary costs to build the production facility.

**Table 18.2.** Summary of the investment for the entire plant, including both the *E. coli* processing and the RBC conversion process.

<b>Investment Summary</b>		
<b><u>Total Bare Module Costs:</u></b>		
Fabricated Equipment	\$	1,813,373
Process Machinery	\$	7,815,631
Spares	\$	-
Storage	\$	251,664
Other Equipment	\$	-
Catalysts	\$	-
Computers, Software, Etc.	\$	95,867
<b><u>Total Bare Module Costs:</u></b>	<b>\$</b>	<b><u>9,976,534</u></b>
<b><u>Direct Permanent Investment</u></b>		
Cost of Site Preparations:	\$	498,827
Cost of Service Facilities:	\$	498,827
Allocated Costs for utility plants and related facilities:	\$	-
<b><u>Direct Permanent Investment</u></b>	<b>\$</b>	<b><u>10,974,188</u></b>
<b><u>Total Depreciable Capital</u></b>		
Cost of Contingencies & Contractor Fees	\$	1,975,354
<b><u>Total Depreciable Capital</u></b>	<b>\$</b>	<b><u>12,949,541</u></b>
<b><u>Total Permanent Investment</u></b>		
Cost of Land:	\$	258,991
Cost of Royalties:	\$	-
Cost of Plant Start-Up:	\$	1,294,954
<b>Total Permanent Investment - Unadjusted</b>	<b>\$</b>	<b>14,503,486</b>
Site Factor		1.00
<b><u>Total Permanent Investment</u></b>	<b>\$</b>	<b><u>14,503,486</u></b>

# **Section 19:**

## **Operating Costs - Cost of Manufacturing**

## 19. Operating Costs

### 19.1. Variable Costs

The purchase of raw materials per ECO RBC transfusion unit is approximately \$43.75, which leads to an annual cost of \$8.75 million. These values are shown in Table 19.1.1.

**Table 19.1.1.** Estimated raw material pricing and their respective yearly costs. The following table includes all materials needed for both the *E. coli* processing and the RBC treatment.

Raw Material	Annual Amount Required		Cost of Raw Material		Cost per RBC Unit	Annual Cost
AGE	2.21	L	\$ 173.00	\$/L	\$ 0.0019	\$ 381.81
Ammonium sulfate	5.20	kg	\$ 51.76	\$/kg	\$ 0.0013	\$ 259.71
APS	0.29	kg	\$ 78.60	\$/kg	\$ 0.0001	\$ 22.45
Beta-Lactose	4.50	L	\$ 463.00	\$/kg	\$ 0.0104	\$ 2,082.28
Chloramphenicol	0.02	kg	\$ 16,905.00	\$/kg	\$ 1.7892	\$ 357,845.04
Detergent	432	kg	\$ 62.60	\$/kg	\$ 0.1352	\$ 27,034.21
DMAAm	16.4	L	\$ 93.00	\$/L	\$ 0.0076	\$ 1,521.26
Ethanol	4154	L	\$ 4.18	\$/L	\$ 0.0868	\$ 17,359.76
Ethylenediamine	78.2	kg	\$ 73.50	\$/L	\$ 0.0320	\$ 6,391.45
Glucose	310	kg	\$ 9.56	\$/kg	\$ 0.0148	\$ 2,968.04
Glutaraldehyde	130	L	\$ 99.50	\$/L	\$ 0.0646	\$ 12,917.37
Glycerol	180	L	\$ 87.50	\$/L	\$ 0.0787	\$ 15,740.76
Guanadine HCl	2428	L	\$ 246.75	\$/L	\$ 2.9957	\$ 599,133.68
HEPES	668	kg	\$ 223.00	\$/kg	\$ 0.0745	\$ 14,901.70
HisPur® Cobalt Resin	50.0	L	\$ 4,980.00	\$/L	\$ 1.2450	\$ 249,000.00
IgG	1.30	g	\$ 790.00	\$/mg	\$ 5.1280	\$ 1,025,600.36
Imidazole	312	kg	\$ 265.00	\$/kg	\$ 0.4137	\$ 82,747.84
IPTG	2.64	kg	\$ 44,800.00	\$/kg	\$ 0.5919	\$ 118,373.71
Kanamycin	0.03	kg	\$ 14,400.00	\$/kg	\$ 0.0022	\$ 448.36
KH <sub>2</sub> PO <sub>4</sub>	675	kg	\$ 137.00	\$/kg	\$ 0.4621	\$ 92,420.75
MBAAm	5.71	kg	\$ 1,044.00	\$/kg	\$ 0.0298	\$ 5,963.54
MES	105	kg	\$ 1,003.00	\$/kg	\$ 0.5285	\$ 105,708.18
Magnesium sulfate	270	kg	\$ 157.00	\$/kg	\$ 0.2118	\$ 42,365.13
Sodium carbonate	55.0	kg	\$ 137.00	\$/kg	\$ 0.0377	\$ 7,541.15
Sodium phosphate heptahydrate	1525	kg	\$ 56.88	\$/kg	\$ 0.4336	\$ 86,711.91
Sodium sulfate	319	kg	\$ 27.92	\$/kg	\$ 0.0446	\$ 8,914.14
Sodium borohydride	9.82	kg	\$ 118.18	\$/kg	\$ 0.0058	\$ 1,160.76
Sodium chloride	2950	kg	\$ 10.38	\$/kg	\$ 0.1532	\$ 30,631.91
Sodium hydroxide	1113	kg	\$ 36.00	\$/kg	\$ 0.2004	\$ 40,072.52
Ammonium chloride	225	kg	\$ 79.00	\$/kg	\$ 0.0888	\$ 17,764.57
Pierce Endotoxin Removal Resin	80.0	L	\$ 20,200.00	\$/L	\$ 8.0800	\$ 1,616,000.00
POROS Benzyl HIC Resin	650	L	\$ 3,800.00	\$/L	\$ 12.3500	\$ 2,470,000.00
Protein A	4.15	kg	\$ 366.00	\$/g	\$ 7.6024	\$ 1,520,484.99
Sodium phosphate	58.0	kg	\$ 42.00	\$/kg	\$ 0.1217	\$ 24,349.93
TEMED	0.25	L	\$ 168.00	\$/L	\$ 0.0002	\$ 41.44
Urea	2146	kg	\$ 67.40	\$/kg	\$ 0.7234	\$ 144,670.03
Total					\$ 43.75	\$ 8,749,530.74

The total cost for raw materials for the process is \$8.75 million. This cost estimate was determined from prices quotes provided by vendors. All reagents are United States Pharmacopoeia grade (USP), as required by the FDA. The resin for the endotoxin affinity chromatography column is the most expensive raw material needed to perform the process, followed by protein A, and guanidine HCl. Although relatively high, it should be noted that numerous steps in the purification processes require costly resin. Additionally, the cryogel-based affinity chromatography column requires expensive chemicals, polymers, and IgG antibodies that drastically increase this cost. Although for the purposes of this design the amount of materials and costs required for this column are reasonable, there are concerns that the possibility of expansion may cause financial strain if antibodies need to be purchased more often.

In addition to the raw materials required for the process, there are a number of disposable products that will be used for each batch. These costs are summarized in Table 19.1.2.

**Table 19.1.2.** Additional variables costs associated with the single-use, disposable technology that is used annually.

Unit	Disposable Unit	Vendor	Cost	Number per Batch	Cost per RBC Unit	Annual Cost
Media Sterilization Filter	Filter	Millipore	\$ 650.00	1	\$ 0.36	\$ 72,800.00
Innoculum Growth Reactor	ambr 250® Vessel	Sartorius	\$ 155.00	1	\$ 0.09	\$ 17,360.00
Microfluidization Product Resuspension Container	500L 3D Bioprocess Container	Thermo Fisher Scientific	\$ 300.00	1	\$ 0.17	\$ 33,600.00
Cobalt Affinity Chromatography Collection Container	500L 3D Bioprocess Container	Thermo Fisher Scientific	\$ 300.00	1	\$ 0.17	\$ 33,600.00
Endotoxin Affinity Chromatography Collection Container	500L 3D Bioprocess Container	Thermo Fisher Scientific	\$ 300.00	1	\$ 0.17	\$ 33,600.00
Endotoxin Affinity Chromatography Collection Container	200L 3D Bioprocess Container	Thermo Fisher Scientific	\$ 250.00	1	\$ 0.14	\$ 28,000.00
HIC Collection Container	50L 2D Labtainer Bioprocess Bag	Thermo Fisher Scientific	\$ 150.00	1	\$ 0.08	\$ 16,800.00
Final Product Holding Container (Enzyme)	5L 2D Labtainer Bioprocess Bag	Thermo Fisher Scientific	\$ 150.00	10	\$ 0.84	\$ 168,000.00
Hyperbaric Chamber Collection Container	200L 3D Bioprocess Container	Thermo Fisher Scientific	\$ 250.00	3	\$ 0.42	\$ 84,000.00
WAVE Bioreactor	200L Flexsafe® RM Bag	Sartorius	\$ 200.00	6	\$ 0.67	\$ 134,400.00
Final Product Holding Container (RBC)	500L 3D Bioprocess Container	Thermo Fisher Scientific	\$ 300.00	1	\$ 0.17	\$ 33,600.00
Blood Bags for Distribution	Terumo Blood Bags	Terumo BCT	\$ 0.90	1786	\$ 0.90	\$ 180,028.80
Total					\$ 4.18	\$ 835,788.80

These include disposable bags for reactors, storage containers, and filters. The total annual cost for these products is \$835,789, which is an order of magnitude smaller than the total annual cost for the raw materials. This also amounts to \$4.18 per RBC unit, of which the cost of the final distribution bag accounts for \$0.90. All costs were obtained from vendors and requested quotes. This result was particularly satisfactory as it justifies the use of single-use technology for all short-term storage. Originally, industrial consultants, particularly Jeffrey Cohen (Johnson & Johnson) and Daniel Green (GlaxoKlineSmith) were concerned that the cost of using single-use technology would outweigh the benefits of reduced CIP/SIP times. Upon performing this financial assessment of the annual costs required, though, single-use technology greatly increases the time with which batches can be processed and greatly reduces the demand for steam in this plant.

## 19.2. Utilities

The total annual cost of utilities is \$580,547, which is shown in Table 19.2.1. The cost per RBC unit is \$2.90 and includes WFI, lighting, HVAC, electricity, refrigeration, and steam costs.

**Table 19.2.1.** Annual utility cost estimates required with the respective costs per ECO RBC transfusion unit made.

Utility	Annual Amount Required		Cost of Utility		Cost per RBC Unit	Annual Cost
Water for Injection	41400	L	0.0050	\$/L	\$ 0.001	\$ 207
Lighting and HVAC	2120000	kWh	0.0516	\$/kWh	\$ 0.547	\$ 109,400
Electricity	2886000	kWh	0.1541	\$/kWh	\$ 2.223	\$ 444,600
Refrigeration	46200	kWh	0.0506	\$/kWh	\$ 0.012	\$ 2,340
Steam	2334	kg	0.0130	\$/kg	\$ 0.012	\$ 2,400
Total					\$ 2.79	\$ 558,947

The WFI is produced by a WFI supplier, which treats tap water to later be used in the process for SIP. WFI is required for all production processes since the facility is built to be pharmaceutical grade. The water required for the WFI priced is based on estimates from the Medford, Massachusetts Department of Public Works, Water and Sewer Division.



Lighting and HVAC requirements were adapted from average values in established literature “Energy Benchmarking in the Pharmaceutical Industry,” assuming a plant size of 6,000 square feet. 10.6 kWh of lighting/HVAC utility are required per unit RBCs. Electricity requirements for each process unit were obtained from manufacturer brochures; 4.81 kWh per unit of RBCs are required to power process equipment. The industrial cost of electricity in Medford, MA, \$0.0514/kWh, was used to determine the cost of electricity for operating all unit operations, lighting, HVAC, and refrigeration. Lighting/HVAC and process electricity requirements are priced at \$0.547/unit RBCs and \$0.74/unit RBCs, respectively.

Refrigeration is required to maintain the cold room, where blood bags for conversion will be organized and stored until they are processed. Each cold room will have dimensions of 30 ft x 30 ft x 8 ft and is maintained at 4°C and 25% relative humidity. Thermal resistance values for wall, ceiling, and floor materials are standard values for cold room building materials provided by North Carolina State University. The service load, which accounts for lighting requirements, cold room control equipment, and storage carts, people, and humid air entering and exiting the cold room, are included in utilities analysis and assumed to be 10% of the heat conduction in the cold room. The total heat load, including heat conduction and service load, is 15,500 kWh per year with a requirement of 0.077 kWh/unit RBCs. The cost of refrigeration per unit product was determined to be \$0.0039/unit RBCs.

Lastly, low pressure steam is required in the process plant to sterilize the stainless steel fermenters and to kill any live mass remaining the bio-waste tank. All cultures or surfaces that were exposed to microbial cultures will be heated to 121°C (see Appendix C) for approximately 11 minutes and then cooled to 80°C. The steam is priced at \$0.013 per kilogram, and it is assumed that the process requires 2,334 kg annually (0.10 grams enzyme per mg enzyme). This leads to a cost of \$0.012/unit RBCs.

### **19.3. Fixed Operating Costs**

The inputs for calculating fixed operating costs are shown in Table 19.3.1. To ensure successful plant operation, it assumed that five daily operating shifts are necessary, each lasting approximately 4.8 hours. It is assumed that there will be two operators for each shift, while there

will be an quality control specialist for three of the five shifts. Although the number of operators is quite high, they are necessary given that there are two separate processes being conducted in parallel.

**Table 19.3.1.** Summary of the inputs for fixed cost analysis.

Fixed Costs		
<u>Operations</u>		
Operators per Shift:	13 (assuming 5 shifts)	
Direct Wages and Benefits:	\$40 /operator hour	
Direct Salaries and Benefits:	15% of Direct Wages and Benefits	
Operating Supplies and Services:	6% of Direct Wages and Benefits	
Technical Assistance to Manufacturing:	\$60,000.00 per year, for each Operator per Shift	
Control Laboratory:	\$65,000.00 per year, for each Operator per Shift	
<u>Maintenance</u>		
Wages and Benefits:	4.50% of Total Depreciable Capital	
Salaries and Benefits:	25% of Maintenance Wages and Benefits	
Materials and Services:	100% of Maintenance Wages and Benefits	
Maintenance Overhead:	5% of Maintenance Wages and Benefits	
<u>Operating Overhead</u>		
General Plant Overhead:	7.10% of Maintenance and Operations Wages and Benefits	
Mechanical Department Services:	2.40% of Maintenance and Operations Wages and Benefits	
Employee Relations Department:	5.90% of Maintenance and Operations Wages and Benefits	
Business Services:	7.40% of Maintenance and Operations Wages and Benefits	
<u>Property Taxes and Insurance</u>		
Property Taxes and Insurance:	2% of Total Depreciable Capital	
<u>Straight Line Depreciation</u>		
Direct Plant:	8.00% of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities	
Allocated Plant:	6.00% of 1.18 times the Allocated Costs for Utility Plants and Related Facilities	
<u>Other Annual Expenses</u>		
Rental Fees (Office and Laboratory Space):	\$3,555,000	
Licensing Fees:	\$0	
Miscellaneous:	\$342,100	
<u>Depletion Allowance</u>		
Annual Depletion Allowance:	\$0	

The complete fixed cost summary is shown in Table 19.3.2. The combination of fixed operating expenses, site maintenance costs, and general yearly expenses totals \$15.2 million. It should be noted that this value is quite high and exceeds the fixed costs associated with certain industrial chemical processes. The reason behind these high costs is most likely associated with having a large number of operators present. Additionally, operating overhead, property taxes and

insurance, and other annual expenses may actually be lower than the assumptions made for this project. However, it was assumed that all these values were useful in calculating an upper bound on what the fixed costs would be, allowing for a strict feasibility analysis.

**Table 19.3.2.** Summary of all fixed costs, including operating expenses required per year for the execution of plant duties, site maintenance, and general expense estimates.

<b><u>Fixed Cost Summary</u></b>		
<b><u>Operations</u></b>		
Direct Wages and Benefits	\$	5,408,000
Direct Salaries and Benefits	\$	811,200
Operating Supplies and Services	\$	324,480
Technical Assistance to Manufacturing	\$	780,000
Control Laboratory	\$	845,000
<b>Total Operations</b>	<b>\$</b>	<b>8,168,680</b>
<b><u>Maintenance</u></b>		
Wages and Benefits	\$	582,729
Salaries and Benefits	\$	145,682
Materials and Services	\$	582,729
Maintenance Overhead	\$	29,136
<b>Total Maintenance</b>	<b>\$</b>	<b>1,340,278</b>
<b><u>Operating Overhead</u></b>		
General Plant Overhead:	\$	493,280
Mechanical Department Services:	\$	166,743
Employee Relations Department:	\$	409,909
Business Services:	\$	514,123
<b>Total Operating Overhead</b>	<b>\$</b>	<b>1,584,055</b>
<b><u>Property Taxes and Insurance</u></b>		
Property Taxes and Insurance:	\$	258,991
<b><u>Other Annual Expenses</u></b>		
Rental Fees (Office and Laboratory Space):	\$	3,555,000
Licensing Fees:	\$	-
Miscellaneous:	\$	342,100
<b>Total Other Annual Expenses</b>	<b>\$</b>	<b>3,897,100</b>
<b><u>Total Fixed Costs</u></b>	<b>\$</b>	<b>15,249,104</b>

# **Section 20:**

## **Economic and Profitability Analysis**

## **20. Economic and Profitability Analysis**

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### **20.1. Market Analysis Flux and Basic Approach**

The service fee applied to the enzymatic treatment of RBC units is based on the current cost for hospitals to collect and treat RBC units from donors. The average cost for RBC units to hospitals has risen over the last 20 years, from \$155 in the early 1990s to \$243.10 in 2016 [25,26]. The price charged to patients is higher, at \$219 in the early 1990s and \$423 in 2016 [25,26]. The enzymatic treatment of RBC units will be an additional service added to the collection and treatment of existing RBC units. The price for this service will be a portion of the cost to collect and treat RBC units and will be charged to hospitals, but ultimately the patient or insurance agency will cover the fee.

Approximately 21 million units of RBCs are needed yearly in the United States and the demand is steadily increasing as the population ages and more surgeries are required [2]. The enzymatic conversion operation aims to reach 200,000 RBC units annually, or almost 1.0% of the national market. This is enough to cover the demand for a large hospital system in the United States. With further development of the process and expansion, a larger proportion of the market could be reached.

### **20.2. Clinical Trial Considerations**

The process for the enzymatic conversion of RBCs for transfusion medicine shows the promise to be both lifesaving and somewhat profitable. No other blood product offers both non-immunogenic properties and large scale production. The large scale production of universal RBCs is revolutionary and could replace traditional packaged RBCs for most applications.

The plant produces 200,000 units of universal RBCs annually and generates \$34,293,532 annual revenue in the third production year. The Return on Investment (ROI) for the plant at the current selling price of \$187.50 is 17.17% and the Internal Rate of Return (IRR) is 21.73%. The Net Present Value (NPV) of this project in 2019 is \$8,461,700.

The price of clinical trials for FDA approval was not included in the profitability analysis. As previously mentioned, the projected cost for Phase I-III clinical trials is approximately \$100-200 million, as advised by Scott Diamond, Ph.D., from the University of Pennsylvania. The scale of this plant is not sufficient to recoup the initial investment required to perform these trials.

However, this plant is large enough to produce the requisite materials necessary to perform the large scale Phase I-III trials and thus could initially operate as a pilot plant. Initial safety studies and Phase I trials of the ECO-B enzyme have been successful, which paves the way for an accelerated Phase I trial [4]. Phase II and III trials will need to be more comprehensive, with larger patient populations [23]. These trials could take additional time, approximately 2-3 years and subsequent approval a year later. However, the design and performance of these trials is out of the scope of this project and will not be included in the profitability analysis.

Nonetheless, it is worth mentioning that the start-up nature of this project, if successful, could make it an enticing partner with large biotechnological and pharmaceutical companies present on the East Coast. Currently, GlaxoKlineSmith, Celgene Corporation, Kite Pharmaceuticals, and Merck have all been developing their blood biology departments to address numerous hematological diseases. If this project is deemed financially feasible, collaborations with these conglomerates could help provide adequate funding to execute clinical trials without imposing further costs. Additionally, it opens up the possibility of mergers with these companies that could help with the recovery of initial investments and lead to additional profitability.

### **20.3. Pricing of ECO RBCs**

Current blood transfusion unit prices tend to average around \$309 in the Boston area. Using this as a basis, the service price for the enzymatic treatment of RBCs to convert them to universal type will be approximately 60% of the purchase cost hospitals pay for transfusion units. This decision was done after performing a sensitivity analysis on the profitability of this venture by varying the selling price of the transfusion unit. The service price for the enzymatically converted RBCs is approximately half of the collection and treatment cost to hospitals. The selling price will \$187.50 per transfusion unit, and it increases by 4.34% up until the year 2024, shown in Table 18.3. Once 2024 begins, however, the price change will only happen in increments of 0.5% for the duration of the facility's production life. The increase in price is to account for the natural market growth in demand for RBC units. Additionally, since it is difficult to protect how other blood collecting organizations will evolve in the next decade, it may be a more realistic approach to stabilize prices after some time to be competitive with other transfusion unit sources. Overall, the price increases by \$34.16 over the duration of the facility's production life.

**Table 20.3.1.** The projected selling price and yearly increase for enzymatically converted RBC units. Up until the year 2024, the transfusion units will see a yearly increase of 4.34% in price, reflecting the most recent trends in the transfusion market. Beginning in 2024, it has been decided that the price will only increase by 0.5% annually.

Year	Manual Input Price	Percent Increase
2021	\$187.50	--
2022	\$195.64	4.34%
2023	\$204.13	4.34%
2024	\$212.99	4.34%
2025	\$214.05	0.50%
2026	\$215.12	0.50%
2027	\$216.20	0.50%
2028	\$217.28	0.50%
2029	\$218.37	0.50%
2030	\$219.46	0.50%
2031	\$220.55	0.50%
2032	\$221.66	0.50%

#### **20.4. Profitability Analysis**

An initial capital investment of \$25.6 million will be required. Table 20.4.1 summarizes the working capital allotted to the project. It is assumed that one year will be needed for finalizing design and an additional year for construction. The plant will be active for 12 years, leading to a total duration of 14 years for the project. A conservative approach was taken and it was assumed that the plant will operate at 90% of the desired production goal of 200,000 ECO RBC transfusion units, beginning from year one.

**Table 20.4.1.** Summary of working capital requirements for the first year of production.

<b>Working Capital</b>					
	<u>2020</u>		<u>2021</u>		<u>2022</u>
Accounts Receivable	\$ 8,321,918	\$	-	\$	-
Cash Reserves	\$ 870,676	\$	-	\$	-
Accounts Payable	\$ (678,199)	\$	-	\$	-
ECO RBCs Inventory	\$ 3,624,658	\$	-	\$	-
Raw Materials	\$ 647,260	\$	-	\$	-
<b>Total</b>	<b>\$ 12,786,313</b>	<b>\$</b>	<b>-</b>	<b>\$</b>	<b>-</b>
<i>Present Value at 15%</i>	<i>\$ 11,118,533</i>	<i>\$</i>	<i>-</i>	<i>\$</i>	<i>-</i>
<b><u>Total Capital Investment</u></b>		<b>\$</b>	<b>25,622,019</b>		

Table 20.4.2 provides an overview of multiple profitability metrics for the process in its third year of production. As previously mentioned, the plant will have an IRR of 21.73%, slightly above the nominal rate of 15%. The ROI is calculated to be 17.17%. The net present value of the project in 2019 is \$8.46 million. The total capital investment involved in the project is \$27.3 million. It should be noted that this process will require a significant amount of research and specialized technology optimization, which will cause future cash flows to be reinvested with return rates even greater than the estimated IRR.

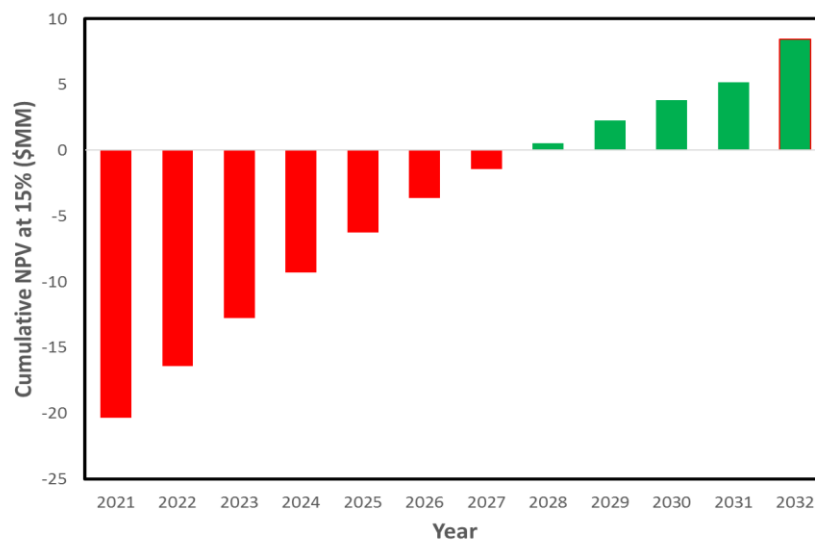
**Table 20.4.2.** Summary of profitability metrics for the RBC conversion plant using a selling price of \$187.50 per ECO RBC transfusion unit.

<b>Profitability Measures</b>	
<b>The Internal Rate of Return (IRR) for this project is</b>	21.73%
<b>The Net Present Value (NPV) of this project in 2019 is</b>	\$ 8,461,700
<b>ROI Analysis (Third Production Year)</b>	
Annual Sales	36,743,070
Annual Costs	(28,146,775)
Depreciation	(1,160,279)
Income Tax	(2,751,326)
Net Earnings	4,684,690
Total Capital Investment	27,289,799
ROI	17.17%



This financial analysis provides some initial insight into the feasibility of the project. Assuming proper partnerships can be established with major hospitals and blood banks, this project is slightly profitable. The successful profit in this analysis is also assuming that the process plant continues to produce only 200,000 ECO RBC transfusions per year. Realistically, it is expected that the start-up will expand to cover the blood demands of other major hospitals in the state of Massachusetts and potentially other nearby metropolitan areas along the East Coast. This implies that after several years of operation, the actual production goal and capacity will increase. Although expansion will also imply increased equipment and operating costs, there will be additional compounded profits. Our equipment selection was also careful to incorporate smaller scale bioreactor towers that can actually handle the operation of more than one seed bioreactor, providing an initial area in which costs will already be lower than expected upon expansion. Once the process has been optimized, industrial consultant Arthur Etchells, Ph.D., states that the number of batches completed in a week. This will naturally increase production capacity without investing in further equipment costs.

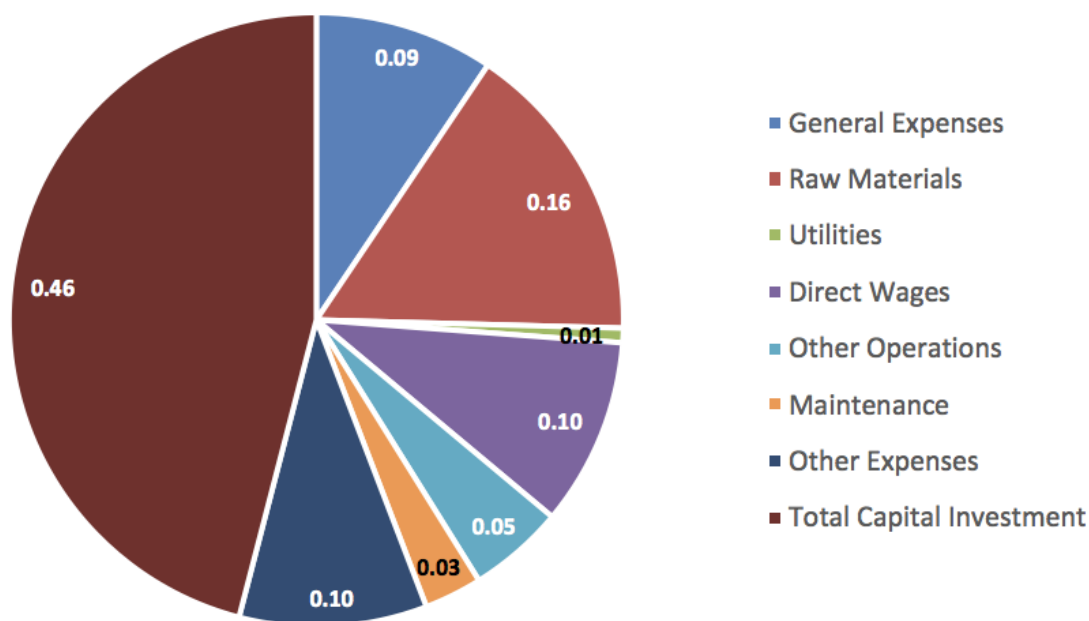
The net present value of the studied base for the process over a production lifetime of 12 years was approximately \$8.46 million with a selling price as described in Section 20.3. A plot of the cumulative cash flow is shown in Figure 20.4.1.



**Figure 20.4.1.** Cumulative discounted free cash flow for project over a twelve year production period. The base selling price of each ECO RBC unit is \$187.50, which increases by 4.34% until 2024 and then only by 0.50% for the rest of the plant’s lifetime.

## 20.5. Sensitivity Analysis

The cost to produce a unit of enzymatically converted RBCs can be broken down into principal components to assess how changes would affect the overall cost. The total capital investment is the largest component of the cost to produce the RBC units, at 46% of the cost. The largest variable in the total capital investment is the bare module factor, which can change based on the nature of the process machinery required for the plant. The next largest section are the raw materials, contributes 16% to the total cost of the RBC units. The raw materials constitute a majority of the variable costs associated with the production of the RBC units. This is followed by direct wages and other expenses as the leading contributors to the cost of RBC unit production.



**Figure 20.5.1:** Distribution of costs per unit of RBCs.

To examine how key factors contribute to the plant's profitability, a sensitivity analysis was performed on the components that comprised the largest segments of the total cost of the RBC unit. Variables, such as product price, variable costs, operator wages, and bare module cost were studied to determine which were most influential on the profitability of the plant. The sensitivity analysis was used to guide the selection of the final values for these variables.

## **20.6. Service Price and Variable Costs**

The service price was studied in relation to the variable costs to determine its effect on the Internal Rate of Return (IRR). The service price was varied between +/- 50% of the initial set service price, which corresponds to approximately half the price of a traditional RBC unit. Additionally, the variable costs were studied for +/- 50% of the set variable cost.

The plant becomes unprofitable below a product price of \$122.50 and has an IRR of 48.54% at a service price of \$262.50, 150% of the set price. The IRR varied from -0.80% to 31.57% as the variable cost ranged from +/- 50% of the set variable cost. While the variable cost does affect the profitability of the plant, the service price has a much larger effect on the profitability. For the set service price of \$175 the plant can be profitable for variable costs ranging from below \$7,165,373 to \$20,063,045. For this reason variable costs were not considered as an important metric for further studies of plant profitability, which is detailed in Figure 5.10.3.3.

**Table 20.6.1:** Internal Rate of Return (IRR) as a function of variable costs and product price.

Product Price	Variable Costs										
	\$7,165,373	\$8,598,448	\$10,031,522	\$11,464,597	\$12,897,672	\$14,330,746	\$15,763,821	\$17,196,895	\$18,629,970	\$20,063,045	\$21,496,119
	\$87.50	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	\$105.00	-6.18%	-11.69%	-17.89%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	\$122.50	6.27%	2.16%	-2.21%	-6.95%	-12.14%	-17.92%	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	\$140.00	15.93%	12.42%	8.80%	5.03%	1.08%	-3.12%	-7.63%	-12.54%	-17.95%	Negative IRR
	\$157.50	24.18%	20.98%	17.73%	14.42%	11.02%	7.52%	3.89%	0.09%	-3.93%	-12.89%
	<b>\$175.00</b>	31.56%	28.55%	25.52%	22.46%	19.36%	16.22%	13.01%	9.73%	6.36%	2.85%
	\$192.50	38.31%	35.44%	32.55%	29.66%	26.75%	23.81%	20.85%	17.86%	14.82%	11.72%
	\$210.00	44.58%	41.81%	39.04%	36.26%	33.48%	30.69%	27.89%	25.07%	22.23%	19.36%
	\$227.50	50.44%	47.76%	45.08%	42.40%	39.72%	37.03%	34.34%	31.65%	28.94%	26.23%
	\$245.00	55.96%	53.36%	50.75%	48.15%	45.55%	42.95%	40.35%	37.75%	35.15%	32.54%
\$262.50	61.18%	58.64%	56.10%	53.57%	51.04%	48.52%	45.99%	43.47%	40.95%	38.43%	
											35.91%

The IRR sharply rises with small changes in the price of the RBC unit service, as shown in Figure 5.10.3.2. The plant has a negative IRR when the service price is set below \$150 per unit of RBCs. The final service cost of \$175 was chosen to maximize profitability and customer requirements. At this value the plant has an internal rate of return of 16.22% and the customer pays a service fee that is approximately half of the original cost of the RBC unit, which was determined previously to be the upper limit for customer satisfaction.



**Figure 20.6.1:** Effect of service price per RBC unit on Internal Rate of Return (IRR)

The effect of variable costs on the IRR is much smaller than that of the service price, but as shown in Figure 5.10.3.3 the profitability of the plant does marginally increase as the variable costs decreases. As the variable costs ranged from \$7 million to \$21.5 million, or +/- 50% of the original variable costs the IRR only increased 32.4%. In order to see increases in profitability, dramatic reductions in the variable costs would have to be made. Because the variable costs didn't greatly influence the profitability as other factors did, optimization efforts were focused elsewhere.

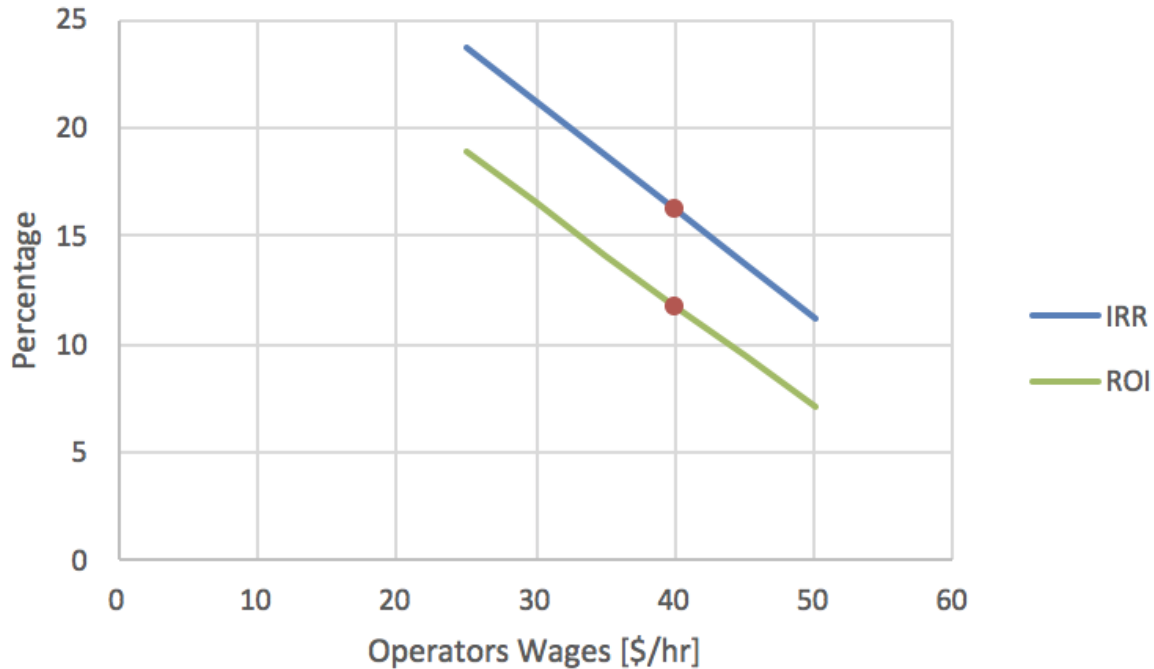


**Figure 20.6.2:** Effect of variable costs on Internal Rate of Return (IRR).

## 20.7. Operator Wages

The operator wages account for \$5,408,000 of the \$28,145,384 annual costs, or 19.2% of the total costs. This is the second largest cost, behind variable costs, excluding the capital investment. Because of this, it is important to determine the effect changes in operator wages have on the profitability of the plant.

As operator wages increased the IRR and ROI decreased linearly, suggesting a strong relationship between profitability and the operator wages. The operator wage of \$40/hour was selected for its acceptable profitability measures and industry competitiveness. The technical nature of the work in this plant and high quality control measures in place raise the operator wage rate. However, even at high wage rates, the plant can be profitable, only becoming unprofitable at wages higher than \$60/hour.

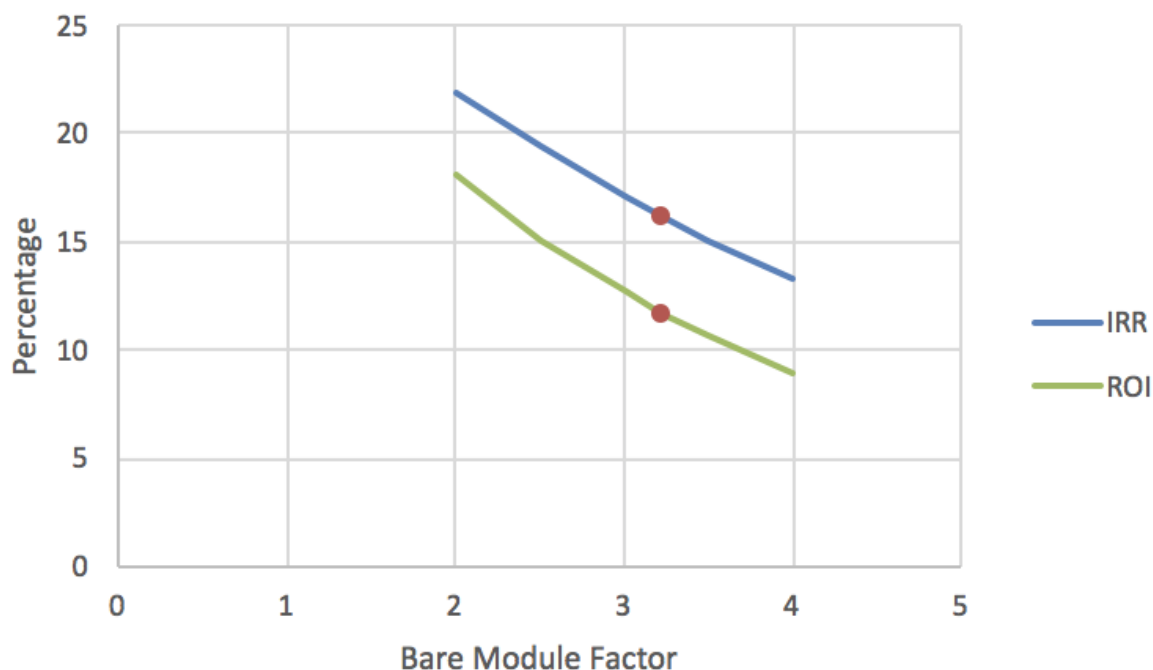


**Figure 20.7.1:** Effect of operator wages on Internal Rate of Return (IRR) and Return on Investment (ROI).

## 20.8. Bare Module Factor

The bare module factor is used to predict the final purchase and installation cost for equipment. It is highly dependent on the nature of the equipment purchased. For instance, machinery that needs to be custom designed, fabricated, and installed incurs a larger bare module cost than equipment purchased off the shelf. Much of the equipment needed for this process falls in the later category, and thus has a lower bare module cost.

The default bare module factor used for most applications is 3.21, however that likely overestimates the bare module cost for this plant. A sensitivity analysis was performed to see how the bare module factor affected the profitability of the plant. This is especially important, because the equipment costs are a majority of the total capital investment.



**Figure 20.8.1:** Effect of Bare Module Factor on the Internal Rate of Return (IRR) and the Return on Investment (ROI).

The bare module factor had a modest effect on both profitability measures. Large changes in the bare module factor showed only about a 20% change in the IRR and ROI values, but corresponds to significant changes in the bare module cost. For the purpose of the profitability analysis a bare module factor of 3.21 was used as an upper bound as further effort to quantify the bare module factor would have a modest effect on the overall plant profitability.

## 20.9. Facility Lifespan

The plant has an operation life expectancy of about 12 years. The anticipated lifespan of the facility accounts for the manufacturers' projected lifespans of the equipment and the projected market for RBCs in general. New methods for producing universal RBCs are being studied, but they will not be viable for large scale production for at least a decade until they begin to collect human clinical trial data [11]. Enzymatically converted RBCs will therefore be the only competitor in this market segment for the lifespan of the facility. If the plant were to shut down before the end of its projected 12-year lifespan, there could be some value in the salvage of the purchased equipment. It is recommended however to operate at full production capacity for its planned 12-year occupancy.



# **Section 21: Other Important Considerations**

## **21. Other Important Considerations**

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### **21.1. Steam-in-Place (SIP) Requirements**

Unless otherwise specified, process equipment will be steam-sterilized in place (SIP) after cleaning in place (CIP) to ensure neutralization of *E. coli*, unconverted A and B RBCs, used ECO-A and ECO-B enzyme, and other biological process waste that could pose harm to the environment or persons if disposed of before neutralization. SIP requires equipment sterilization with saturated steam at 50 psi and 138°C to ensure that all equipment reaches 121°C for 11 minutes, complying with FDA sterilization regulations.<sup>51</sup> Flow of room temperature air through equipment tubing will be used to cool the equipment post-SIP. Cooling times for equipment are according to manufacturer brochures and/or equipment size.

### **21.2. Clean-in-Place (CIP) Requirements**

Any and all equipment that processes cells, enzyme, or other biological material must be treated as biological waste or cleaned according to FDA clean-in-place (CIP) regulations. Non-disposable equipment that contacts biological materials will be cleaned according to CIP protocol between batches; CIP is accounted for on all process scheduling Gantt charts.

CIP for reusable process equipment will consist of a rinse with water for injection (WFI), followed by two detergent flushes and an intermediate rinse with WFI. An acidic solution will be used to ensure neutralization of all bio-waste and clearance of all protein and cell residue. A rinse with WFI to rid the equipment of any remaining detergent and an air blow over the equipment to remove moisture complete CIP. The amounts of detergent and acid solution required annually for CIP and, correspondingly, the costs of these materials, are assumed negligible as compared to other raw material costs in the economic analysis for the process.

### **21.3. Waste Treatment and Environmental Concerns**

All live cells and cell cultures, particularly *E. coli*, may grow or accumulate in water supply or elsewhere and become a hazard to the surrounding environment. Thus, it is critical to inactivate *E. coli* cultures before disposal. For a 12-log reduction in *E. coli* population size, it was assumed

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<sup>51</sup> Effective Bactericidal Treatment , 21 C.F.R. Sect. 1240 (2017).

that *E. coli* must be treated at 121°C for 5 minutes.<sup>52</sup> A D-value (thermal deactivation coefficient) of 0.4167 minutes, the time required for one decimal reduction of the population, was calculated based on this assumption. The number of cells to be inactivated was calculated from batch volumes and final cell concentrations for ECO-A enzyme and ECO-B enzyme production assuming first-order deactivation kinetics. To more thoroughly ensure cell death, it is optimal to achieve a 25-log reduction in cell population; a thermal inactivation time of 10.4 minutes for both ECO-A and ECO-B *E. coli* cells was calculated based on this criterion and final cell concentrations. Live cultures will thus be heated to 121°C for 10-11 minutes to ensure cell death (see Appendix D). Any process units that contact live cells or other biological materials will be autoclaved or otherwise thoroughly sterilized. Waste transport and disposal may be contracted to a nearby facility in Cambridge due to the costly nature of waste handling equipment for a startup venture.

In addition to CIP/SIP practices, hygienic conditions will be met for equipment and operating personnel with quality materials and ventilation. Quality assessment may be performed at various points in the process to assure batch uniformity; quality control in the form of limulus amebocyte lysate (LAL) assay, flow cytometry, or thin layer chromatography will be performed on the final product before packaging and shipping. Current good manufacturing practice (cGMP) protocols will be strictly followed at every step of the process.

#### **21.4. Water for Injection (WFI) Concerns**

It is critical that all water for injection (WFI) generated for process operations be maintained at the proper pH, have no microbial colonies, and contain no particulate matter. Thus, the temperature of the WFI distribution at peak load will be maintained at 80°C. The WFI still will be monitored daily for microbes. WFI samples with sizes of 100-300 mL per test will be taken from various points in the supply loop for 20 working days. Tests for compliance with FDA specifications for WFI will be taken over the course of one year to monitor changes in the WFI chemical composition over time. Tests for residual chlorine, conductivity, and pH will be performed on the WFI year-round. All growth media must pass growth promotion testing.

In addition to the routine testing for microbial growth previously described, the presence of microbes in the WFI will be tested using a membrane filtration technique called total

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<sup>52</sup> Osaili T et al. Thermal inactivation studies of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ready-to-eat chicken-fried beef patties. *Journal of Food Protection* 2006: 69(5):1080–1086.

heterotrophic plate count. WFI will be plated using plate count agar to perform testing for presence of microbiological agents; any colonies present on the agar filter will be identified and further tested for potential safety concerns. For WFI to be considered safe according to FDA standards, plate counts must be less than 10 CFU/mL.

Finally, sanitary clamped piping, instruments, and valves will be used to transport WFI to distribution and storage systems. Piping and instruments will be labeled, properly sloped for optimal flow, and maintained to pass integrity testing. A backflow valve will be installed with the still to protect source water.

### **21.5. Intellectual Property Concerns and FDA Approval**

Consideration of intellectual property concerns prompts consideration of the possibility of licensing the process to a research institution that has conducted studies on enzymatic conversion of RBCs. In addition, a Biologic License Application (BLA) for production, distribution, and medical usage of ECO-RBCs would need to be filed with the FDA before plant startup. A BLA requires extensive pre-clinical tests and successful passage of Phase I-III clinical trials. Pre-clinical tests would study ECO-RBCs for their stability in storage, morphology, biochemistry, oxygen dissociation, and hemolytic properties as compared to those of natural type O RBCs. Clinical trials in human patients would examine the immunogenicity, circulatory half-life, hemoglobin clearance, and blood clearance rate of ECO-RBCs relative to those of wild type O RBCs. Indication selection of patients for clinical trial would need to be performed very carefully. While Phase I clinical trials may be conducted in healthy patients, Phase II and III clinical trials would need to be conducted with patients with measurable indications. Patients with indications such as traumatic bleeding would not be selected, as obtaining informed consent is difficult and transfusion challenging to control. Instead, patients with anemia or undergoing elective surgery would be chosen to participate in clinical trial as response to WT RBCs is well-established and obtainment of informed consent is easier. Consultation with Scott Diamond, Ph.D, of the Department of Chemical and Biomolecular Engineering at the University of Pennsylvania led to an estimated cost of \$100-\$200 million for FDA approval of ECO-RBCs. While this cost is considered out of scope for the purposes of this project, it is vital to consider if the plant were to be realized.

## **21.6. Alternative Applications**

The primary alternative application of production of ECO-RBCs to use in hospitals and distribution to blood banks for use in clinical application is production of ECO-RBCs to provide a more universal blood supply to the US military. The high demand for blood products, particularly universal RBCs and plasma for transfusion, on the battlefield could lead to defense funding for ECO-RBC production if this application were to be pursued.

# **Section 22:**

## **Conclusions and Recommendations**

## **22. Conclusions and Recommendations**

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The design of a start-up-scale plant that produces the glycosidases for RBC conversion and treats RBC units to create universal RBC units shows promise for profitability and expansion. The plant requires an initial capital investment of \$25.6 million and will operate over a 12 year lifespan. During this time the plant will be able to produce 200,000 converted RBC units, enough to satisfy the needs of a single hospital system near the plant's location in Medford, MA. The start-up-scale plant will have a 21.73% internal rate of return over its lifespan, and can maintain profitability over a variety of operating conditions. The plant breaks even in its eighth year of operation, and opportunities for expansion could enhance the profitability of this process.

The profitability of this plant does not account for the expected cost of clinical trials and FDA approval, which is estimated to be \$100-200 million. The high cost of the clinical trials makes the start-up-plant unprofitable, however, the scale of this plant was not designed to recover the initial costs of FDA approval. Instead, the start-up-scale plant is designed to be a proof of concept and to produce enough converted RBC units to supply the Phase I-III clinical trials necessary for approval. Once the blood product is approved for use in humans, the process could be expanded beyond the start-up-scale plant. Large scale central production of the glycosidases could be optimized for distribution to local treatment facilities. Under this expansion regime, the process could provide universal RBCs to east coast cities with relative logistical ease and could recover the initial capital investment required for approval.

Due to the high profitability of the start-up-scale plant, projected FDA approval, and the potential for further expansion, it is recommended that this venture is pursued. The enzymatic conversion of RBCs has the potential to alleviate many of the growing concerns in the blood market, including blood wastage and incompatibility reactions. However, investors should be mindful of the risks associated with the blood market. Shifts in the demand for different types of blood products and the growing efficiency of surgeries could affect the demand for RBC units, however, the scale of the process is not likely to ever exceed this demand.

# **Section 23:**

# **Acknowledgements**



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### **Heat Inactivation and Endotoxin Removal**

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# **Abstract**

## Appendix A: Cell Growth Analysis

### Assumptions:

1. Prepared media begins with a glucose concentration of 2 g/L in all fermenters and bioreactors.
2. During the exponential growth phase, there is negligible cell death. Therefore, the specific growth rates is defined as:  $\mu_{net} = u_g = 0.25 \text{ hr}^{-1}$ .
3. All glucose consumption is due to metabolic consumption by the cells.
4. The batch bioreactor is well mixed at all times. Therefore, the cell, nutrient, and oxygen concentrations are uniform throughout the bioreactors' contents.
5. Cell loss due to optical density testing of inoculum, growth, and production bioreactors is considered negligible. The only loss that is not negligible is accounted for in the mass balance during transfer out procedures.
6. Oxygen level is held constant in the production bioreactor to ensure aerobic cellular respiration throughout the growth period.

### Cell Growth Mathematical Modeling:

Equation (3) was used to determine all batch times required to achieve desired final cell concentrations at the end of each bioreactor/fermentation step. The mathematical manipulation of equation (3) is as follows:

$$\frac{dX}{X} = \mu_{net} dt$$

$$\int_{X_0}^X \frac{dX}{X} = \int_0^t \mu_{net} dt = \mu_{net} \int_0^t dt$$

$$t = \frac{1}{\mu_{net}} \int_{X_0}^X \frac{dX}{X}$$

$$t = \frac{1}{\mu_{net}} \ln \frac{X}{X_0} \quad (A.1)$$

Where:  $t$  = time (hours)

$X_0$  = initial cell concentration (cells/mL)

$X$  = cell concentration at time  $t$

$\mu_{net}$  = specific growth rate for cell culture ( $\text{hr}^{-1}$ )

Initial and final cell concentrations for the various steps taken in the process were obtained from literature on high-density microbial cultures.

#### A. For inoculum culture:

$$t = \frac{1}{0.25 \text{ hr}^{-1}} \ln \frac{4.42 \times 10^8}{5.09 \times 10^6} = 17.86 \text{ hr}$$

Recommended growth times for *E. coli* inoculum growth range between 12 to 16 hours, which validates this batch time calculation.

#### B. For growth bioreactor:

We assume that 5% of the cells cultured in the previous step are either lost or lysed, implying that 95% of the previous culture inoculates the new bioreactor.

For the culture to produce  $\alpha$ -N-acetylgalactosaminidase:

$$t = \frac{1}{0.25 \text{ hr}^{-1}} \ln \frac{3.45 \times 10^9}{8.50 \times 10^6} = 24.03 \text{ hr}$$

For the culture to produce  $\alpha$ -galactosidase:

$$t = \frac{1}{0.25 \text{ hr}^{-1}} \ln \frac{3.17 \times 10^9}{8.50 \times 10^6} = 23.69 \text{ hr}$$

**C. For production fermenters:**

We assume that 5% of the cells cultured in the previous step are either lost or lysed, implying that 95% of the previous culture inoculates the new fermenters.

It was recommended from the literature that the initial concentration of *E. coli* in the fermenter be adjusted to  $4.50 \times 10^7$  cells/mL.

$$t = \frac{1}{0.25 \text{ hr}^{-1}} \ln \frac{2.91 \times 10^{10}}{4.50 \times 10^7} = 25.89 \text{ hr}$$

## Appendix B: Required Protein Yield to Satisfy Production Goals

### Assumptions:

All suggestions to account for losses were provided by Robert Luo, Ph.D. from GlaxoKlineSmith.

1. 200,000 universal RBC transfusion units will be prepared. Following the blood-type distribution in the United States, nearly two-thirds of these prepared units will come from type-A blood and the remaining one-third will from type-B.
2. Type-AB conversions will not be considered for this process since they make up such a small percentage (<3%) of the donor population.
3. During RBC conversion treatment/processing, 35% of the RBCs will be lost. To satisfy the RBC cell count in each blood bag, 307,693 RBC units are needed to account for losses and meet production goals.
4. 40% of each enzyme product will be lost in downstream purifications steps.
5. 10% of the enzyme product will not be functional due to protein misfolding, denaturation, or possible points mutations that altered amino acid sequence and affect functionality.
6. One unit of packed RBCs is 300 mL.

### Required mass yield of $\alpha$ -galactosidase:

$$307,693 \text{ units} \times 0.67 \frac{\text{type} - A \text{ units}}{\text{total units}} \times 60 \frac{\text{mg}}{\text{type} - A \text{ unit}} \times \frac{1 \text{ g}}{1000 \text{ mg}} \times \frac{1}{1 - 0.4} \times \frac{1}{0.9}$$
$$= 22,906 \text{ g of } \alpha - \text{galactosidase}$$

### Required mass yield of for $\alpha$ -N-acetylgalactosaminidase:

$$307,693 \text{ units} \times 0.33 \frac{\text{type} - A \text{ units}}{\text{total units}} \times 2 \frac{\text{mg}}{\text{type} - A \text{ unit}} \times \frac{1 \text{ g}}{1000 \text{ mg}} \times \frac{1}{1 - 0.4} \times \frac{1}{0.9}$$
$$= 376.07 \text{ g of } \alpha - N - \text{acetylgalactosaminidase}$$



## Appendix C: Biowaste System Design

### Assumptions:

1. The final cell concentration in the production culture is  $2.91 \times 10^{10}$  cells/mL. Upon removal from the process, the *E. coli* bacteria will be inactivated in the waste vessel.
2. Inactivation kinetics are first order.
3. The thermal deactivation coefficient is  $D = 0.4167$  minutes. A literature review was performed to determine what the decimal reduction time for *E. coli* at  $121^\circ\text{C}$  was. Numerous sources reported different values. Therefore, an average value was taken for these inactivation times.

Using the production of  $\alpha$ -galactosidase as the base case with which to determine the time for thermal inactivation of *E. coli* since it requires a larger processing volume, the calculations the time to reach the thermal death point are as follows:

$$2.91 \times 10^{10} \frac{\text{cells}}{\text{mL}} \times 396 \frac{\text{L}}{\text{batch}} \times 1000 \frac{\text{mL}}{\text{L}} = 4.16 \times 10^{16} \frac{\text{cells to inactivate}}{\text{batch}}$$

First order inactivation kinetics:

$$\ln\left(\frac{N}{N_0}\right) = -kt \quad (C.1)$$

Where:  $N$  = microbial population at any time,  $t$

$N_0$  = initial microbial population

$D$  = decimal reduction time; time required for a 1-log (or 10-fold) cycle reduction in the microbial population

The thermal inactivation constant is defined as:

$$k = \frac{2.303}{D}$$

Manipulating equation (B.1), the following expression for thermal inactivation is obtained:

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \quad (C.2)$$

To determine heating time required for thermal inactivation:

$$t = -D \cdot \log\left(\frac{25 - \log \text{reduction population size}}{\text{Number of cell to inactivate}}\right) \quad (C.3)$$

$$t = -0.4167 \text{ min} \cdot \log\left(\frac{4.16 \times 10^{-9}}{4.16 \times 10^{16}}\right)$$

$$t = 10.42 \text{ minutes}$$

Therefore, bio-waste tank will be heated to 121°C and maintained at this temperature for approximately 11 minute to inactivate bacteria.

**Conclusion:** Thus, there is  $4.16 \times 10^{-9}$  chance that a single *E. coli* cell will be active after an eleven minute heat inactivation in each batch.

## Appendix D: Batch Sizing

### Assumptions:

1. The process to produce bacterial glycosidases is designed to run 114 batches per year: 112 for the production of  $\alpha$ -N-acetylgalactosaminidase and 2 for  $\alpha$ -galactosidase. The plant will be operational for 24 hours, 7 days a week, 52 weeks per year. Six batches will be completed each week. Although this exceeds calculated operation times, it provides additional time to account for possible errors or malfunctions that may arise.
2. Accounting for losses and possible inactive enzyme, the ultimate goal is to produce 22.9 kg of  $\alpha$ -N-acetylgalactosaminidase and 0.376 kg of  $\alpha$ -galactosidase per year.
3. The specific growth rate for BL21(DE3) recombinant *E. coli* cells is  $0.25 \text{ hr}^{-1}$ .
4. 20% of the inoculum died from both the freezing and thawing processes.
5. 5% of the cell population in the growth and production bioreactors will be dead or inactive.
6. Frozen stock of *E. coli* will be prepared at concentration of  $7.95 \times 10^6$  cells/mL.
7. Oxygen limitations will not be a concern for the growth periods and will be held constant using PID control at 40% dissolved  $\text{O}_2$ .

All values were validated by Jeffrey Cohen, Ph.D. from GlaxoKlineSmith to confirm similarity to commonly observed values in industrial fermentation.

### For the production of $\alpha$ -N-acetylgalactosaminidase

**Table D.1. Inoculum batch sizing details for  $\alpha$ -N-acetylgalactosaminidase production.** The parameters for the predicted growth model were obtained from literature review. All final cell concentrations were obtained from a report on high density microbial cultures.

Inoculum Growth Bioreactor		
Working volume	0.110	L
Initial cell concentration	$5.09 \times 10^6$	cells/mL
Specific growth time	0.25	$\text{hr}^{-1}$
Batch time	17.86	hr
Target cell concentration	$4.42 \times 10^8$	L

**Table D.2. Growth bioreactor batch sizing details for  $\alpha$ -N-acetylgalactosaminidase production.** The parameters for the predicted growth model were obtained from literature review. All final cell concentrations were obtained from a report on high density microbial cultures. It was assumed that five percent of the inoculum culture was lost due to cell death or transfer inefficiencies when inoculating the seed bioreactor.

Growth Bioreactor		
Working volume	5.44	L
Initial cell concentration	$8.50 \times 10^6$	cells/mL
Specific growth time	0.25	hr <sup>-1</sup>
Batch time	24.03	hr
Target cell concentration	$3.45 \times 10^9$	L

**Table D.3. Production fermentation batch sizing details for  $\alpha$ -N-acetylgalactosaminidase production.** The parameters for the predicted growth model were obtained from literature review. All final cell concentrations were obtained from a report on high density microbial cultures. Again, five percent of the seed bioreactor culture was assumed lost due to cell death or transfer efficiencies. In this step, the culture from the seed bioreactor was equally split between two stainless steel fermenters to achieved productions goals. During IPTG induction, the temperature of the reactor was lowered to 25°C to inhibit cell growth.

Production Fermenters		
Working volume	396	L
Initial cell concentration	$4.50 \times 10^7$	cells/mL
Specific growth time	0.25	hr <sup>-1</sup>
Batch time	25.89	hr
Target cell concentration	$2.91 \times 10^{10}$	cells/mL
Protein production rate	85.4	ng/cell-day
Protein production time	5	hr
Protein produced	0.205	kg

### **For the production of $\alpha$ -galactosidase**

**Table D.4. Inoculum batch sizing details for  $\alpha$ -galactosidase production.** The parameters for the predicted growth model were obtained from literature review. All final cell concentrations were obtained from a report on high density microbial cultures.

<b>Inoculum Growth Bioreactor</b>		
Working volume	0.110	L
Initial cell concentration	$5.09 \times 10^6$	cells/mL
Specific growth time	0.25	hr <sup>-1</sup>
Batch time	17.86	hr
Target cell concentration	$4.42 \times 10^8$	L

**Table D.5. Growth bioreactor batch sizing details for  $\alpha$ -galactosidase production.** The parameters for the predicted growth model were obtained from literature review. All final cell concentrations were obtained from a report on high density microbial cultures. It was assumed that five percent of the inoculum culture was lost due to cell death or transfer inefficiencies when inoculating the seed bioreactor. The final cell concentration in this seed bioreactor is slightly less for  $\alpha$ -galactosidase production than for  $\alpha$ -galactosidase production since the volume of culture is less to satisfy production goals.

<b>Growth Bioreactor</b>		
Working volume	5.44	L
Initial cell concentration	$8.50 \times 10^6$	cells/mL
Specific growth time	0.25	hr <sup>-1</sup>
Batch time	23.69	hr
Target cell concentration	$3.17 \times 10^9$	L

**Table D.6. Production fermentation batch sizing details for  $\alpha$ -galactosidase production.** The parameters for the predicted growth model were obtained from literature review. All final cell concentrations were obtained from a report on high density microbial cultures. Again, five percent of the seed bioreactor culture was assumed lost due to cell death or transfer efficiencies. In this step, the culture from the seed bioreactor was equally split between two stainless steel fermenters to achieved productions goals. During IPTG induction, the temperature of the reactor was lowered to 25°C to inhibit cell growth.

<b>Production Fermenters</b>		
Working volume	364	L
Initial cell concentration	$4.50 \times 10^7$	cells/mL
Specific growth time	0.25	hr <sup>-1</sup>
Batch time	25.89	hr
Target cell concentration	$2.91 \times 10^{10}$	cells/mL
Protein production rate	85.6	ng/cell-day
Protein production time	5	hr
Protein produced	0.189	kg

## Appendix E: Culture Media Composition

**Table E.1.** High-salt medium will be used for high-density microbial fermentation. This composition has been experimentally determined to promote optimal cell growth to OD<sub>600</sub> values up to 45, which corresponds to a cell concentration of  $3.6 \times 10^{10}$  cells/mL.

High-Mineral Salt Microbial Culture Media	
Component	Concentration
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O	33.9 g/L
KH <sub>2</sub> PO <sub>4</sub>	15.0 g/L
NH <sub>4</sub> Cl	5.0 g/L
NaCl	2.5 g/L
Na <sub>2</sub> SO <sub>4</sub>	7.1 g/L
MgSO <sub>4</sub>	6.0 g/L
Glycerol	0.4 % v/v
β-lactose	0.01 % v/v

## Appendix F: Oxygen Transfer Limitations for Reactor Design

### Assumptions:

1. The final cell concentration in the production culture is  $2.91 \times 10^{10}$  cells/mL.
2. Specific uptake rate of oxygen,  $q_{O_2}$ , is 12.0 mmole  $O_2$ /g cells-hr for *Escherichia coli*.
3. The difference between the saturated oxygen solubility,  $C_O^*$ , and the actual concentration of dissolved oxygen in the culture broth,  $C_O$ , is 1.13 mmole  $O_2$ /L.
4. For the chosen bioreactor and impeller configurations, the volumetric oxygen transfer coefficient,  $k_L a$ , is 308 hr<sup>-1</sup>.

To find the maximum cell density at which the production bioreactor can provide enough oxygen to maintain cell growth and viability, the oxygen uptake rate (OUR) was set equal to the oxygen transfer rate (OTR).

### **OTR calculation:**

$$OTR = k_L a (C_O^* - C_O) \quad (F.1)$$

$$OTR = 308 \text{ hr}^{-1} \left( 1.13 \frac{\text{mmole } O_2}{L} \right)$$

$$OTR = 348.04 \frac{\text{mmole } O_2}{L - \text{hr}}$$

### **Maximum cell density:**

$$OUR = q_{O_2} X = OTR \quad (F.2)$$

$$X = \frac{OTR}{q_{O_2}} = \frac{348.04 \frac{\text{mmole } O_2}{L - \text{hr}}}{12.0 \frac{\text{mmole } O_2}{g \text{ cells} - \text{hr}}}$$

$$X = 29.0 \frac{g \text{ cells}}{L}$$



## Appendix G: Endotoxin Yield Calculations

### Assumptions:

1. There is  $1.00 \times 10^{-10}$  grams of endotoxin for every  $10^5$  *E. coli* cells in the final culture.
2. The final cell concentration in the production fermenters is  $2.91 \times 10^{10}$  cells/mL. It is further assumed that all endotoxin production is accounted for in the final cell concentration.

### **For $\alpha$ -N-acetylgalactosaminidase production:**

Batch volume:  $V = 396$  L

$$\frac{1.0 \times 10^{-10} \text{ g endotoxin}}{10^5 \text{ cells}} \cdot \frac{2.91 \times 10^{10} \text{ cells}}{\text{mL}} \cdot \frac{1000 \text{ mL}}{1 \text{ L}} \cdot 396 \text{ L} = 11.5 \text{ g endotoxin}$$

### **For $\alpha$ -galactosidase production:**

Batch volume:  $V = 364$  L

$$\frac{1.0 \times 10^{-10} \text{ g endotoxin}}{10^5 \text{ cells}} \cdot \frac{2.91 \times 10^{10} \text{ cells}}{\text{mL}} \cdot \frac{1000 \text{ mL}}{1 \text{ L}} \cdot 364 \text{ L} = 10.6 \text{ g endotoxin}$$

## Appendix H: Cobalt Affinity Chromatography Sizing and Timing

The cobalt affinity chromatography column size and volumes of solution needed were calculated according to the manufacturer's protocol for the HisPur® cobalt affinity resin. The given binding capacity of the resin (20 grams of protein per liter of resin) and the mass of either  $\alpha$ -galactosidase or  $\alpha$ -N-acetylgalactosaminidase entering the column were used to calculate the volume of resin required per batch. This calculation assumes that all enzyme that passes through the column will bind to the resin and disregards the 90% recovery from the unit. This assumption ensures that recovery is not limited by the binding capacity of the resin. The void fraction of the resin packaging is 0.30 according to the manufacturer's protocol. The column volume was calculated as follows:

$$\text{Resin Volume} = \frac{\text{Enzyme mass into the column (g)}}{\text{batch}} \cdot \text{Binding Capacity} \quad (\text{H.1})$$

**For  $\alpha$ -N-acetylgalactosaminidase:**

$$205 \frac{\text{g}}{\text{batch}} \cdot \frac{1 \text{ L resin}}{20 \text{ g protein}} = 10.25 \frac{\text{L resin}}{\text{batch}}$$

$$\text{Required column volume to process one batch} = \frac{\text{Resin Volume}}{\text{batch}} \cdot \frac{1}{1 - \text{Void Fraction}} \quad (\text{H.2})$$

$$10.25 \text{ L resin} \cdot \frac{1}{1 - 0.30} = 14.6 \text{ L}$$

The data sheet for the resin allows for 25 regenerations before resin must be replaced. The amount of resin needed for processing  $\alpha$ -N-acetylgalactosaminidase on an annual basis was calculated as follows:

$$\frac{\text{Total volume of resin required}}{\text{year}} = \frac{\text{Resin}}{\text{Batch}} \cdot \frac{\text{Batches}}{\text{year}} \cdot \frac{1}{\text{Number of Uses before Discarding}} \quad (\text{H.3})$$

$$\text{Total resin volume needed} = 10.25 \frac{\text{L resin}}{\text{batch}} \cdot 112 \text{ batches} \cdot \frac{1}{25} = 45.92 \text{ L resin}$$

**For  $\alpha$ -galactosidase:**

$$189 \frac{g}{batch} \cdot \frac{1 L resin}{20 g protein} = 9.45 \frac{L resin}{batch}$$

$$Required\ column\ volume\ to\ process\ one\ batch = \frac{Resin\ Volume}{batch} \cdot \frac{1}{1 - Void\ Fraction}$$

$$9.45 L resin \cdot \frac{1}{1 - 0.30} = 13.5 L$$

Assuming that we use new resin when switching over to the production of  $\alpha$ -galactosidase, the calculations for the total amount of resin needed on an annual basis for processing  $\alpha$ -galactosidase.

$$\frac{Total\ volume\ of\ resin\ required}{year} = \frac{Resin}{Batch} \cdot \frac{Batches}{year} \cdot \frac{1}{Number\ of\ Uses\ before\ Discarding}$$

$$Total\ resin\ volume\ needed = 9.45 \frac{L resin}{batch} \cdot 2 batches \cdot \frac{1}{25} = 0.756 L resin$$

From these calculations, the total volume of resin required to treat both enzymes is approximately 46.7 liters. An additional conclusion that can be made is that since the mass of  $\alpha$ -N-acetylgalactosaminidase that will be processed is greater than the mass of  $\alpha$ -galactosidase, its minimal required will satisfy the volumetric requirements for both enzymes.

A conservative approach was used in calculating the batch time and amount of buffer needed by using the maximum column volumes of buffer suggested in the protocol.

**Table H.1. Calculated Material Requirements for Chromatography Buffers.** Compositions and volumes required were obtained from the HisPur® Superflow Agarose resin vendor sheet. Masses required were calculated using the maximum protocol volumes listed.

Buffer Composition	Protocol Volumes	Composition	Amount per Batch
<b>Denaturing/Equilibrium</b>	3x-10x column volume	5mM Imidazole 20mM Sodium Phosphate 300mM sodium chloride	Imidazole 1.70 kg Sodium Phosphate 1.44 kg
<b>Wash</b>	6x-10x column volume	15mM Imidazole 20mM Sodium Phosphate 300mM sodium chloride	Sodium Chloride 7.70 kg MES 0.571 kg
<b>Elution</b>	4x-10x column volume	150mM Imidazole 20mM Sodium Phosphate 300mM sodium chloride	Guanidine HCl 16.8 kg
<b>Regeneration</b>	10x column volume	20mM MES	Detergent 0.293 kg
<b>CIP</b>	2x column volume 5x column volume water	6M guanidine HCl 1% nonionic detergent	Ultrapure Water 689 L

$$Column\ run\ time = Volume\ of\ water \cdot \frac{1}{linear\ flow\ rate \cdot column\ cross\ section} \quad (H.4)$$

$$Column\ run\ time = \frac{689,000\ mL}{150 \frac{cm}{hr} * (14\ cm)^2 * \pi} = 7.46\ hr$$

## Appendix I: Hydrophobic Interaction Chromatography Sizing and Timing

The hydrophobic interaction chromatography column size and volumes of solution needed were calculated according to the protocol for the POROS Benzyl HIC Resin. Note that endotoxin here is denoted as LPS. The given binding capacity of the resin ( $2 \times 10^6$  endotoxin units per mL of resin) and the mass of endotoxin entering the column were used to calculate the volume of resin required per batch. This unit disregards any endotoxin loss that may have occurred in the past few steps. Random packing was assumed with a void fraction of 0.40 as no other data could be found in the literature. The column volume was calculated as follows:

$$\text{Resin Volume} = \frac{\text{Endotoxin mass into the column (g)}}{\text{batch}} \cdot \text{Binding Capacity} \quad (I.1)$$

**For  $\alpha$ -N-acetylgalactosaminidase:**

$$11.5 \frac{\text{g LPS}}{\text{batch}} \cdot \frac{1 \text{ mL resin}}{2 \times 10^6 \text{ EU}} \cdot \frac{1 \text{ L resin}}{1000 \text{ mL resin}} \cdot \frac{1 \text{ EU}}{1 \times 10^{-10} \text{ g LPS}} = 57.5 \frac{\text{L}}{\text{batch}}$$

$$\text{Required column volume to process one batch} = \frac{\text{Resin Volume}}{\text{batch}} \cdot \frac{1}{1 - \text{Void Fraction}} \quad (I.2)$$

$$57.5 \text{ L} \cdot \frac{1}{1 - 0.40} = 95.8 \text{ L}$$

The data sheet for the resin allows for 10 regenerations before resin must be replaced. The amount of resin needed on an annual basis was calculated as follows:

$$\frac{\text{Total volume of resin required}}{\text{year}} = \frac{\text{Resin}}{\text{Batch}} \cdot \frac{\text{Batches}}{\text{year}} \cdot \frac{1}{\text{Number of Uses before Discarding}} \quad (I.3)$$

$$\text{Total resin volume needed} = 57.5 \frac{\text{L resin}}{\text{batch}} \cdot 112 \text{ batches} \cdot \frac{1}{10} = 644 \text{ L resin}$$

**For  $\alpha$ -galactosidase:**

$$10.6 \frac{g \text{ LPS}}{\text{batch}} \cdot \frac{1 \text{ mL resin}}{2 \times 10^6 \text{ EU}} \cdot \frac{1 \text{ L resin}}{1000 \text{ mL resin}} \cdot \frac{1 \text{ EU}}{1 \times 10^{-10} \text{ g LPS}} = 52.9 \frac{\text{L}}{\text{batch}}$$

$$\text{Required column volume to process one batch} = \frac{\text{Resin Volume}}{\text{batch}} \cdot \frac{1}{1 - \text{Void Fraction}}$$

$$52.9 \text{ L resin} \cdot \frac{1}{1 - 0.40} = 88.2 \text{ L}$$

Assuming that we use new resin when switching over to the production of  $\alpha$ -galactosidase the calculations for the total amount of resin needed on an annual basis for processing  $\alpha$ -galactosidase.

$$\frac{\text{Total volume of resin required}}{\text{year}} = \frac{\text{Resin}}{\text{Batch}} \cdot \frac{\text{Batches}}{\text{year}} \cdot \frac{1}{\text{Number of Uses before Discarding}}$$

$$\text{Total resin volume needed} = 52.9 \frac{\text{L resin}}{\text{batch}} \cdot 2 \text{ batches} \cdot \frac{1}{10} = 10.6 \text{ L resin}$$

A conservative approach was used in calculating the batch time and amount of buffer needed, by using the maximum column volumes of buffer suggested in the protocol.

**Table I.1. Calculated Material Requirements for Chromatography Buffers.** Compositions and volumes required were obtained from the POROS Benzyl HIC Resin vendor sheet. Masses required were calculated using the maximum protocol volumes listed.

Buffer Composition	Protocol Volumes	Composition	Amount per Batch	
<b>Denaturing/Equilibrium</b>	3x-10x column volume	10mM Sodium Phosphate 8M Urea	Sodium Phosphate	11.3 kg
<b>Wash</b>	5x column volume	0.5M sodium chloride	Sodium Chloride	15.2 kg
			Sodium Hydroxide	20.8 kg
			Urea	99.8 kg
<b>Elution</b>	10x column volume	50mM Sodium Phosphate 1.7M Ammonium Sulfate	Ammonium Sulfate	0.234 kg
			Guanidine HCl	119 kg
			Detergent	2.08 kg
<b>Regeneration</b>	3x-5x column volume	1M Sodium Hydroxide	Ultrapure Water	3,532 L
<b>CIP</b>	2x column volume 2x column volume water	6M guanidine HCl 1% nonionic detergent		

$$\text{Column run time} = \text{Volume of water} * \frac{1}{\text{linear flow rate} * \text{column cross section}} \quad (I.4)$$

$$\text{Column run time} = \frac{3,532,200 \text{ mL}}{150 \frac{\text{cm}}{\text{hr}} * (40 \text{ cm})^2 * \pi} = 4.68 \text{ hr}$$

## Appendix J: Endotoxin Affinity Chromatography Sizing and Timing

The endotoxin affinity chromatography column size and volumes of solution needed were calculated according to the manufacturer's protocol for the Pierce High Capacity Endotoxin Removal Resin. Note that endotoxin here is denoted as LPS. The given binding capacity of the resin ( $2 \times 10^6$  endotoxin units per mL of resin) and the mass of endotoxin entering the column were used to calculate the volume of resin required per batch. The void fraction of the resin packaging is 0.40 according to the manufacturer's protocol. The column volume was calculated as follows:

$$\text{Resin Volume} = \frac{\text{Endotoxin mass into the column (g)}}{\text{batch}} \cdot \text{Binding Capacity} \quad (\text{J.1})$$

**For  $\alpha$ -N-acetylgalactosaminidase:**

$$1.15 \frac{\text{g LPS}}{\text{batch}} \cdot \frac{1 \text{ mL resin}}{2 \times 10^6 \text{ EU}} \cdot \frac{1 \text{ L resin}}{1000 \text{ mL resin}} \cdot \frac{1 \text{ EU}}{1 \times 10^{-10} \text{ g LPS}} = 5.75 \frac{\text{L}}{\text{batch}}$$

$$\text{Required column volume to process one batch} = \frac{\text{Resin Volume}}{\text{batch}} \cdot \frac{1}{1 - \text{Void Fraction}} \quad (\text{J.2})$$

$$5.75 \text{ L} \cdot \frac{1}{1 - 0.40} = 9.58 \text{ L}$$

The data sheet for the resin allows for 10 regenerations before resin must be replaced. The amount of resin needed on an annual basis was calculated as follows:

$$\frac{\text{Total volume of resin required}}{\text{year}} = \frac{\text{Resin}}{\text{Batch}} \cdot \frac{\text{Batches}}{\text{year}} \cdot \frac{1}{\text{Number of Uses before Discarding}} \quad (\text{J.3})$$

$$\text{Total resin volume needed} = 5.75 \frac{\text{L resin}}{\text{batch}} \cdot 112 \text{ batches} \cdot \frac{1}{10} = 64.4 \text{ L resin}$$



**For  $\alpha$ -galactosidase:**

$$1.06 \frac{g \text{ LPS}}{batch} \cdot \frac{1 \text{ mL resin}}{2 \times 10^6 EU} \cdot \frac{1 \text{ L resin}}{1000 \text{ mL resin}} \cdot \frac{1 \text{ EU}}{1 \times 10^{-10} \text{ g LPS}} = 5.29 \frac{L}{batch}$$

$$\text{Required column volume to process one batch} = \frac{\text{Resin Volume}}{batch} \cdot \frac{1}{1 - \text{Void Fraction}}$$

$$5.29 \text{ L resin} \cdot \frac{1}{1 - 0.40} = 8.82 \text{ L}$$

Assuming that we use new resin when switching over to the production of  $\alpha$ -galactosidase, the calculations for the total amount of resin needed on an annual basis for processing  $\alpha$ -galactosidase.

$$\frac{\text{Total volume of resin required}}{year} = \frac{\text{Resin}}{Batch} \cdot \frac{\text{Batches}}{year} \cdot \frac{1}{\text{Number of Uses before Discarding}}$$

$$\text{Total resin volume needed} = 5.29 \frac{L \text{ resin}}{batch} \cdot 2 \text{ batches} \cdot \frac{1}{10} = 1.06 \text{ L resin}$$

A conservative approach was used in calculating the batch time and amount of buffer needed, by using the maximum column volumes of buffer suggested in the protocol.

**Table J.1. Calculated Material Requirements for Chromatography Buffers.** Compositions and volumes required were obtained from the Pierce High Capacity Endotoxin Removal Resin vendor sheet. Masses required were calculated using the maximum protocol volumes listed.

Buffer Composition	Protocol Volumes	Composition	Amount per Batch	
<b>Equilibrium</b>	5x column volume	10mM Sodium Phosphate	Sodium Phosphate	0.141 kg
<b>Elution</b>	1-2x column volume	20mM Sodium Phosphate	Sodium Hydroxide	0.383 kg
			Ethanol	2.10 kg
<b>Regeneration</b>	5x column volume	0.2M Sodium Hydroxide in 95% ethanol	Sodium Chloride	5.60 kg
			Guanidine HCl	11.0 kg
<b>Wash</b>	5x column volume 5x column volume water	2M Sodium Chloride	Detergent	0.192 kg
			Ultrapure Water	278 L
<b>CIP</b>	2x column volume 5x column volume water	6M guanidine HCl 1% nonionic detergent		

$$Column\ Run\ time = Volume\ of\ water * \frac{1}{linear\ flow\ rate * column\ cross\ section} \quad (J.4)$$

$$Column\ run\ time = \frac{499,090\ mL + 55,600\ mL}{150 \frac{cm}{hr} * (20\ cm)^2 * \pi} = 3.01\ hr$$

## Appendix K: Endotoxin Level Calculation

According to both manufacturer's protocols, there is a 99% removal of endotoxin using the POROS Benzyl HIC Resin and the Pierce High Capacity Endotoxin Removal Resin. However, literature values report that the removal is actually closer to 90% in practice for the POROS Benzyl HIC Resin. Thus, we assume that after each pass, 90% of the endotoxins are removed through the hydrophobic interaction column and 99% through the endotoxin affinity chromatography column. To be more stringent on the purity of our final product, we also assume that the ultrafiltration/diafiltration step does not change the endotoxin concentration in the product solution. This assumption is valid given that the endotoxins and the enzymes are all similar in molecular weight and that there no filtration units that can provide decent separation. We also assume that the product solution will undergo two passes through a bulk filtration step to further remove any remaining endotoxins and impurities, each with an assumed 99% removal. Lastly, we for the design of the chromatography columns, we assume that none of the endotoxin that was produced in the original *E. coli* microbial culture that was introduced into downstream purification has been lost. This is done to ensure that the chromatography column is designed to handle the highest possible amount of endotoxin mass to treat. The final product solution will have a concentration of 1 mg/mL.

A sample calculation for the production of  $\alpha$ -N-acetylgalactosaminidase, which has a higher endotoxin mass yield from the *E. coli* culture, is shown. The mass yield of  $\alpha$ -N-acetylgalactosaminidase is 205 grams per batch.

$$11.5 \frac{g \text{ LPS}}{\text{batch}} \cdot 0.10 \cdot 0.01 \cdot 0.01 \cdot 0.01 \cdot \frac{1 \text{ EU}}{10^{-10} g \text{ LPS}} \cdot \frac{1}{205,000 \text{ mL}} = 0.056 \frac{\text{EU}}{\text{mL}}$$

This final values complies with FDA limitations.

## Appendix L: Cryogel-based Affinity Chromatography Sizing and Timing

The material composition of the cryogel used in the column is outlined in Table L.1. The preparation of the affinity matrix by coupling of protein A to the cryogel packed into the chromatography is outlined in Table L.2. Lastly, Table L.3 gives the composition of the storage solution used to preserve the cryogel in the column.

**Table L.1.** Materials required for the synthesis of the cryogel used in the affinity chromatography of RBCs.

<b>Cryogel Composition</b>	
<b>Material</b>	<b>Amount</b>
Degassed water	100 mL
DMAAm	6.3 mL
AGE	0.85 mL
MBAAm	2.2 g
APS	110 mg
TEMED	95 $\mu$ L
<b>Total volume</b>	<b>107.245 mL</b>

**Table L.2.** Materials required for the coupling of protein A to the cryogel.

<b>Protein A Cryogel Coating Composition</b>	
<b>Component</b>	<b>Reagent:Gel ratio</b>
Water	86 L water/L gel
Ethanol	14 L EtOH/L gel
Na <sub>2</sub> CO <sub>3</sub>	2 moles/L gel
Ethylenediamine	5 moles/L gel
Sodium phosphate	1 moles/L gel
Glutaraldehyde	0.5 L glutaraldehyde/L water
Protein A	16 g protein A/L gel
Sodium borohydride	1 moles/L gel
Water	40 L water/L gel

**Table L.3.** Composition of the storage solution used to preserve the cryogel.

<b>Cryogel-Protein A Complex Storage Solution</b>	
<b>Component</b>	<b>Reagent:Gel ratio</b>
Sodium phosphate	1 moles/L gel
Ethanol	2 L EtOH/L gel
Water	8 L water/L gel

Given that the cryogel-based affinity chromatography column was originally used to handle B lymphocytes from whole blood, a volumetric ratio was determined between B lymphocytes and RBCs to approximate what volume of RBCs the column could be treated via this cell separation technique. In this mathematical approach, lymphocytes are modeled as spheres and RBCs as cylinders. The radii are 7  $\mu\text{m}$  and 4  $\mu\text{m}$ , respectively. The height of an RBC is approximately 2  $\mu\text{m}$ . This assumption is valid given that the blood that the RBC treatment process handles only contains RBCs whereas the blood sample in the literature that was used in the column only had B lymphocytes. Neither sample contains additional cell populations that may affect the volumetric ratio.

**B lymphocyte volume:**

$$V_{lymphocyte} = \frac{4}{3}\pi r^3 \quad (L. 1)$$

$$V_{lymphocyte} = \frac{4}{3}\pi(7\mu\text{m})^3 = 1,437 \mu\text{m}^3$$

**RBC volume:**

$$V_{RBC} = \pi r^2 h \quad (L. 2)$$

$$V_{RBC} = \pi(4\mu\text{m})^2(2\mu\text{m}) = 100.5 \mu\text{m}^3$$

The fold-difference between the two is:

$$\frac{V_{lymphocyte}}{V_{RBC}} = 14.3$$

The current plant design expects to produce three batches of universal RBCs per week, for a total of 156 batches throughout the year. To be conservative, it is assumed that no RBCs have been lost yet due to the previous unit operations. This maximizes the amount of cryogel that needs to be prepared, providing the upper bound on cost estimates. The amount of cryogel required to treat one batch of RBCs under this set up is:

$$\frac{\text{Cryogel volume}}{\text{batch}} = \frac{\text{Number of RBCs to be treated per batch}}{\frac{\# \text{ RBCs processed}}{\text{mL gel}}} \quad (L.3)$$

$$\frac{\text{Cryogel volume}}{\text{batch}} = \frac{307,693 \text{ RBC units} \cdot 0.3 \frac{\text{L blood}}{\text{RBC unit}} \cdot \frac{5 \cdot 10^{12} \text{ RBCs}}{1 \text{ L blood}} \cdot \frac{1}{156 \text{ batches}}}{\frac{3 \cdot 10^{10} \text{ lymphocytes}}{\text{mL gel}} \cdot \frac{V_{lymphocyte}}{1 \text{ lymphocyte}} \cdot \frac{14.3 V_{RBC}}{V_{lymphocyte}} \cdot \frac{1 \text{ RBC}}{V_{RBC}}}$$

$$\frac{\text{Cryogel volume}}{\text{batch}} = \frac{2.96 \times 10^{15} \frac{\text{RBCs}}{\text{batch}}}{4.29 \times 10^{11} \frac{\text{RBCs}}{\text{mL gel}}}$$

$$\frac{\text{Cryogel volume}}{\text{batch}} = 6,896 \text{ mL} \approx 6.90 \text{ L}$$

It is recommended that the cryogel occupy below 50% of the total column volume. Therefore:

$$\text{Required column volume to process one batch} = \frac{\text{Cryogel volume per batch}}{1 - 0.5} \quad (L.4)$$

$$\frac{6.90 \text{ L}}{1 - 0.50} = 13.8 \text{ L}$$

We assume that the cryogel complex can be used five times before requiring replacement. Therefore, the total volume of cryogel needed for the 156 planned batches of production is:

$$\frac{\text{Total volume of cryogel required}}{\text{year}} = \frac{\text{Cryogel volume}}{\text{Batch}} \cdot \frac{\text{Batches}}{\text{year}} \cdot \frac{1}{\text{Number of Uses before Discarding}} \quad (L.5)$$

$$\text{Total resin cryogel needed} = 6.90 \frac{\text{L cryogel}}{\text{batch}} \cdot 156 \text{ batches} \cdot \frac{1}{5} = 215 \text{ L cryogel}$$

A conservative approach was used in calculating the batch time and amount of buffer needed, by using the maximum column volumes of buffer suggested in the protocol.

**Table L.1. Calculated Material Requirements for Chromatography Buffers.** Compositions and volumes required were obtained from the HisPur<sup>®</sup> Superflow Agarose resin vendor sheet. Masses required were calculated using the maximum protocol volumes listed.

Buffer Composition	Protocol Volumes	Composition	Amount per Batch	
<b>Denaturing/Equilibrium</b>	20x column volume	0.1M PBS	See Appendix L, Tables L.1 and L.2 for all reagents required  Ultrapure Water 2,123 L	
<b>Wash</b>	6x-10x column volume	0.1M PBS		
<b>Elution</b>	---	Applies mechanical stress.		
<b>Regeneration</b>	10x column volume	50mM acetate		
<b>CIP</b>	2x column volume 5x column volume water	6M guanidine HCl 1% nonionic detergent		

$$\text{Column Run time} = \text{Volume of water} * \frac{1}{\text{linear flow rate} * \text{column cross section}} \quad (L.6)$$

$$\text{Column run time} = \frac{2,123,000 \text{ mL}}{300 \frac{\text{cm}}{\text{hr}} * (14 \text{ cm})^2 * \pi} = 11.5 \text{ hr}$$

## **Appendix M: Utility Requirements for the Refrigerated Room**

### Assumptions:

1. Conduction was assumed to obey Fourier's law.
2. Thermal resistance values (R-values) were adapted from values given in a paper titled "Design of Cooling Facilities: Structure and Energy Requirements"; the R-values used are for standard cold room construction materials.
3. The heat load for the cold room was determined as the amount of heat that would travel through the walls, floor, and ceiling due to conduction.
4. An outside temperature of 21°C (69.8°F) was assumed, as the cold room is located in the interior of the facility.
5. Service load, or field heat, accounting for lighting requirements, equipment requirements, and transport of equipment, laborers, and humid air into and out of the cold room, was assumed to be 10% of the total heat conduction

Three separate cold rooms are required for this process design: one for the downstream purification of the enzymes, another for the RBC treatment process, and a third to store pre-converted and converted RBC transfusion units. The three processes will be kept separate from each other to prevent any type of cross-contamination. For design purposes, it is assumed that all three cold rooms will have the same dimensions. The size of the cold room is estimated to be 900 ft<sup>2</sup>, with dimensions of 30 ft x 30 ft x 8 ft (W x D x H). Total energy requirement was calculated by summing heat conduction and service load. The energy requirement in kWh was calculated assuming 365 days/year of cold room operation. Cold room refrigeration calculations are summarized in Table M.1.



**Table M.1.** All parameters required to calculate the utilities requirements for the designed cold rooms. .

<b>Cold Room Dimensions</b>	
Room Width	30 ft
Room Depth	30 ft
Room Height	8 ft

<b>Temperature Conditions</b>	
Inside Temperature	39.2 °F
Outside Temperature	69.8 °F
Temperature Difference	30.6 °F

<b>R-Values</b>	
Walls	16 sq. ft-°F-hr/BTU
Ceiling	20 sq. ft-°F-hr/BTU
Floor	11 sq. ft-°F-hr/BTU

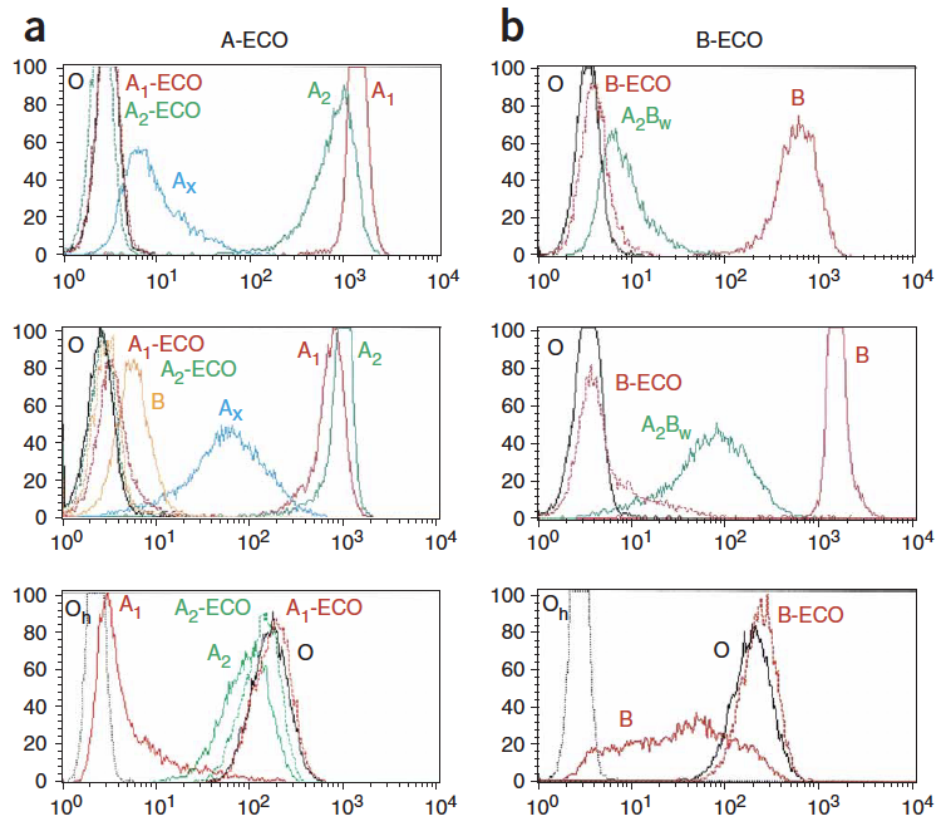
<b>Heat Conduction</b>	
Walls	1836 BTU/hr
Ceiling	1377 BTU/hr
Floor	2504 BTU/hr

<b>Total Heat Calculations</b>	
Total without service load	5717 BTU/hr
Total service load	572 BTU/hr
Total heat	6288 BTU/hr

<b>Price Calculations</b>	
Price of Electricity	0.0514 (\$/kWh)
Total Heat	1.843 kW
Total Power Requirement	16145 kWh
Total Cost of Refrigeration	830 dollars

## Appendix N: Flow Cytometry Quality Control Analysis Representation

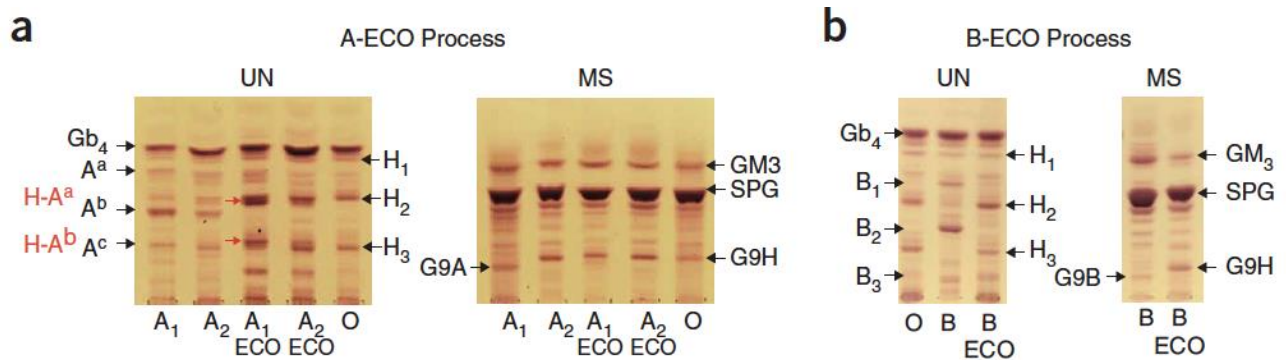
Flow cytometry will be employed to determine cleavage of A and B antigens from ECO-A and ECO-B RBCs produced. It also counts the number of individual cells and register their respective signal intensities. This data will be analyzed using FloJo® FACS software. The information provided by flow cytometry should show that <1% of the treated RBC population have a signal corresponding to blood group antigens. If a sample fails this quality control test, the batch will either be re-treated or discarded.



**Figure N.1. Expected flow cytometry quality control data for (A) ECO-A and (B) ECO-B RBCs.** Flow cytometry measures signal intensity given off by antibodies conjugated with fluorophores. Anti-blood Group A and anti-blood group B antibodies will be mixed with enzymatically converted RBC units for one hour at room temperature to allow for specific binding to blood group antigens. Successful conversion will be determined by signal similarities to pure type-O blood cells – only background noise should be registered. Graphically, this is represented as all the A- and B-ECO signals overlapping with the signal registered from type O-cells.

Sensitive FACS/flow cytometry analysis may be conducted with anti-A and anti-B antibodies. Analysis using FDA-licensed Anti-A blood group reagents should demonstrate total cleavage of A antigen from ECO-A RBCs; similarly, FDA-licensed anti-B reagents should not react or cleave antigen from ECO-B RBCs. Flow cytometry performed with anti-H antibody should show exposure of the H antigen on ECO-A and ECO-B RBCs at similar levels to WT O RBCs. ECO-A RBCs should then have characteristic peaks that align with those of native type-O RBCs, while any A and B control RBCs should have peaks characteristic of A and B RBCs.

## Appendix O: TLC Quality Control Analysis Representation



**Figure O.1.** Representative image of expected results of TLC/SDS-PAGE Western blot analysis of (a) ECO-A and (b) ECO-B RBC glycolipid extracts.

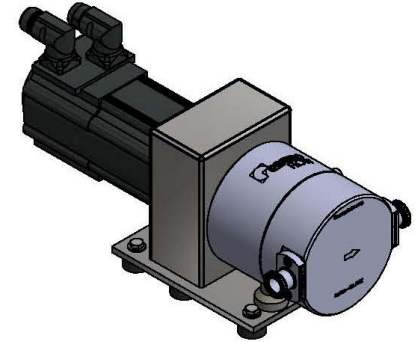
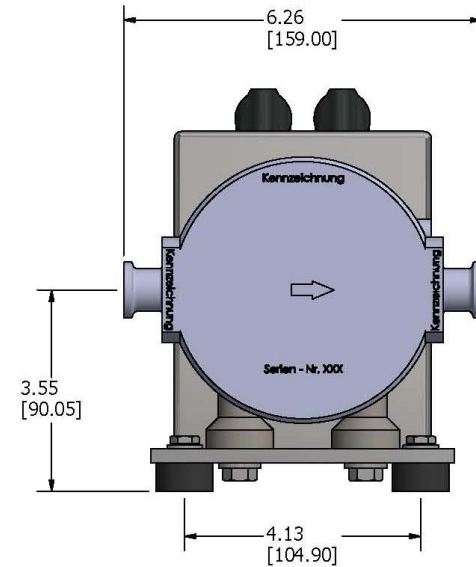
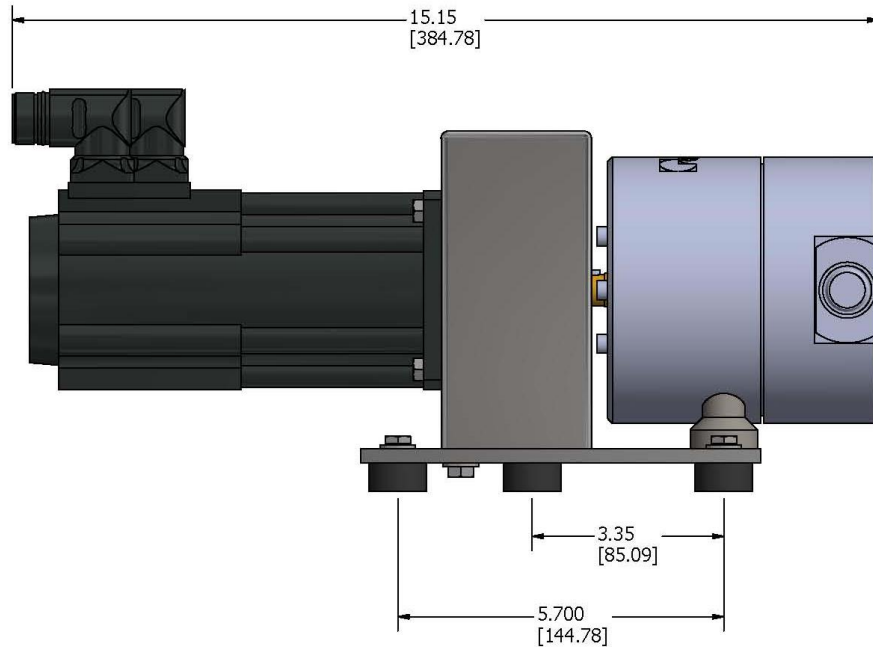
Thin layer chromatography (TLC) and SDS-PAGE Western blot will be conducted as additional quality control tests on treated RBCs' glycobiochemistry (sugar groups). (A) Western blot analysis of glycoprotein extracts from enzymatically converted cells should demonstrate no presence of glycolipids active in A antigen (A<sup>a</sup>, A<sup>b</sup>, and A<sup>c</sup>) and exposure of glycolipids common to native O RBCs (H<sub>1</sub>, H<sub>2</sub>, and H<sub>3</sub>). (B) Similarly, ECO-B RBC glycolipid extracts should only show expression of O glycolipids, not B glycolipids (B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>).

## Appendix P: Adsol-1 (AS-1) Composition

**Table P.1: Composition of Adsol-1 (AS-1) preservative solution that will be used to package and store RBCs.** AS-1 consists of two major components: anticoagulant reagents that prevent agglutination and additional reagents that increase RBC shelf life. This preservative solution allows RBCs to be stored for 42 days at 4°C. The full name of the preservative solution is CPDA-1 Anticoagulant Citrate Phosphate Dextrose Adenine Solution USP.

<b>CPDA-1 Anticoagulant Solution USP</b>		
<b>Anticoagulant and Preservatives</b>	<b>Components</b>	<b>Concentration</b>
	Sodium Citrate (dihydrate)	26.3 g/L
	Dextrose (monohydrate)	25.5 g/L
	Citric Acid (anhydrous)	3.27 g/L
	Monobasic Sodium Phosphate (monohydrate)	2.22 g/L
<b>AS-1 additives</b>	Sodium Chloride	0.900 g/L
	Mannitol	0.750 g/L
	Adenine	0.275 g/L

## Appendix S: Quattroflow Quaternary Diaphragm Blueprints



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# **Appendix:**

## **Literature Sources & Prompt**

# Cell separation using cryogel-based affinity chromatography

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**In cell affinity chromatography, type-specific cell separation is based on the interaction between cell-surface receptors and an immobilized ligand on a stationary matrix. This protocol describes the preparation of monolithic polyacrylamide and polydimethylacrylamide cryogel affinity matrices that can be used as a generic type-specific cell separation approach. The supermacroporous monolithic cryogel has highly interconnected large pores (up to 100  $\mu\text{m}$ ) for convective migration of large particles such as mammalian cells. In this protocol, they are functionalized to immobilize a protein A ligand by a two-step derivatization of epoxy-containing cryogel monolith (reaction with ethylenediamine and glutaraldehyde). Target cells were labeled with specific antibodies and then they were captured in the cryogel through affinity with protein A. These specifically captured cells were recovered in high yields while retaining their viability by mechanical squeezing of the spongy and elastic cryogel matrices. The suggested cell separation protocol takes < 30 min for complete separation on a preprepared protein A-immobilized cryogel column.**

## INTRODUCTION

Preparative cell separation is an important tool to provide a highly purified population of specific cell type for diagnostic, biotechnological and biomedical applications<sup>1</sup>. However, challenges remain associated in the development of effective methods for cell separation on a large scale. An effective cell separation technique should deliver high purity and yield while maintaining cell function. There are two basic approaches to separate cells. The first type is based on physicochemical properties such as size (centrifugal elutriation and density gradient centrifugation), density (density gradient centrifugation), light scattering properties (cell sorter), membrane potential, pH, electrical impedance and charge (aqueous two-phase system and free-flow electrophoresis)<sup>1–3</sup>. Methods of the first type are commonly used for prepurification and concentration, but have low specificity and are difficult to process on a large scale.

The second approach is more specific, is based on affinity interactions, and uses cell-surface molecules. Methods that follow this approach include cell isolation on affinity matrices<sup>4,5</sup>. In flow cytometry, target cells are labeled with an immunofluorescent tag. The technique is accurate but can be time consuming and expensive<sup>6–8</sup>. Similarly, magnetic bead separation technology<sup>9–11</sup> has received attention within the fields of biomedical sciences. Automated magnetic cell sorters are available that can process up to 50 ml of sample volume, but the process is quite expensive and different affinity matrices are required for the separation of different cells. Other cell separation methods have also been developed, such as aqueous two-phase systems<sup>12,13</sup> that are based on specific partition of cells in two immiscible aqueous polymer solutions. This technique can be used for cell separation on a preparative scale, but it requires repetitive extractions of target product to achieve desirable selectivity.

Cell affinity chromatography provides an efficient method for preparative-scale cell separation. The main requirement for cell affinity chromatography is the design of a suitable matrix. Hence, chromatography columns can be either bead packed or monolithic.

## Bead-packed chromatography column

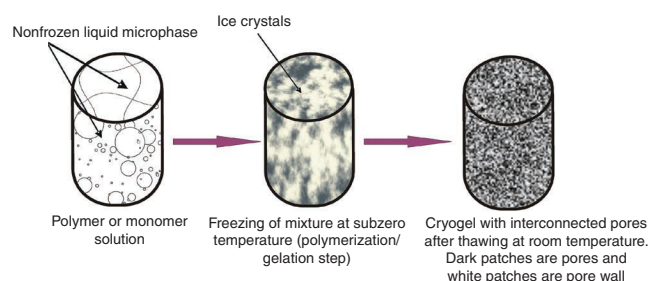
The chromatography column is comprised of granular beads of similar or varying sizes. Fluidized bed chromatography, e.g., expanded bed affinity adsorption (EBA), has shown great promise for a preparative-scale cell separation technique, in which the stationary phase is in the form of a fluidized bed made up of spherical beads<sup>14,15</sup>. In one such example, an immunoaffinity EBA technique was used for the separation of monocytes from human peripheral blood<sup>15</sup>. The limitations associated with this technology have been high shear stress for cells, long equilibration time, requirements for large special columns, narrow range of flow velocity and detachment of bound cells. Furthermore, conventional chromatographic adsorbents such as agarose<sup>16</sup> or silica<sup>17</sup>, used in EBA, can cause nonspecific interactions. The technique also suffers from the inherent limitations of bead-packed chromatography, which include the relatively time-consuming column-packing process and the slow rate of diffusion of solutes within the pores of the bead matrix. Hence, there has been an essential need for designing suitable chromatographic matrix or adsorbents and an efficient approach for the preparative cell separation process.

## Monolithic chromatography columns

These columns have a continuous matrix rather than beads with porous channels. The desired ways of designing such columns can be the development of macroporous chromatographic matrices with interconnected pores that are large enough (much bigger than the cell size), allowing unhindered movement of large particles such as mammalian cells. Moreover, chromatographic matrices should govern the convective mass transport of particles, which is possible if the columns are monolithic.

Therefore, new methods are desirable for the production of hydrophilic macroporous material with a sufficiently large pore size by displacing soluble polymeric chains from a porous network to the walls of pores so that there will be high local polymer concentration in the pore walls (compared with hydrophilic non-macroporous gels). This high local polymer concentration





**Figure 1** | Cryogelation process: polymer or monomer is mixed in aqueous solvent and then the whole system is incubated at subzero temperature for cryopolymerization and/or gelation, along with ice crystal formation (ice crystals function as a porogen). After complete gelation, and when incubated at room temperature and washed with water to remove unreacted monomers or polymeric precursors, the ice crystals melt and leave behind large interconnected pores.

in the pore walls provides an increasing mechanical stability of the material. Such materials known as supermacroporous monolithic 'cryogels' have been developed by carrying out gelation or polymerization at subzero temperature under frozen conditions.

### Supermacroporous monolithic cryogels

The process of cryotropic polymerization or gelation<sup>18</sup> has been established for the development of macroporous hydrophilic gels known as cryogels. Although the polymeric nature of cryogel-type materials has been under study throughout the past decade, their potential for biotechnological applications has been explored only in recent years<sup>19</sup>. Here, cryogels are specially designed from hydrophilic polymers for technologically challenging separation processes such as cell separation.

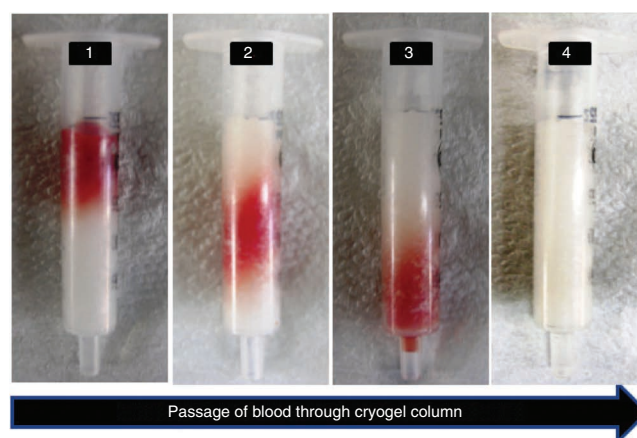
Cryotropic gelation or polymerization is a process whereby under frozen conditions, most of the solvent is frozen, and the dissolved solutes (monomers or polymer precursors) get concentrated in small unfrozen regions called nonfrozen liquid microphase, in which the polymerization or gelation proceeds. After complete synthesis, the melting of solvent crystals (ice, in the case of aqueous media) that function as porogen leaves behind a system of large, continuously interconnected pores. These pores function as capillaries and provide channels for the flow of the mobile phase (Fig. 1). The initial concentration of precursors in solution, their physicochemical properties and the freezing conditions determine the pore size of the cryogel. In contrast to traditional hydrogels, cryogels have a system of interconnected macropores and, because of the structure of pore walls formed as a result of high polymer concentration in nonfrozen liquid microphase, provide relatively higher mechanical strength. High polymer density within pore walls retards solvent diffusion that causes poor swelling and ensures elasticity of cryogels, which allows the flow of liquid at high flow rates with minimal flow resistance. The physical strength of cryogels largely depends on the polymer type and concentration and temperature during cryogel formation. The concentration of polymer in gel phase is higher in cryogels than in conventional gels prepared by other preparation methods. It demonstrates the higher mechanical stability of cryogels in spite of their macroporous morphology.

Cryogels are suitable for the chromatography of bioparticles and cells. Inert cryogel matrices have been produced from

different hydrophilic gel-forming polymers either by polymerization of the appropriate monomers (e.g., polyacrylamide and poly(dimethylacrylamide)) or by physical gelation (e.g., agarose). For bioseparation applications, these matrices provide the convective flow of the solution through the pores wherein the mass transfer resistance is practically negligible. Cryogel matrices also provides sufficiently high flow-through characteristics; the flow rate of water through the monoliths has been estimated in our laboratory at a hydrostatic pressure of 0.01 MPa and has shown a linear flow rate of 2,000–2,500 cm h<sup>-1</sup>. These gels have been designed and used by our group for a variety of challenging separation applications in a monolithic chromatographic mode, including cell separations. The application of these cryogels in the area of cell separation is discussed in the following section.

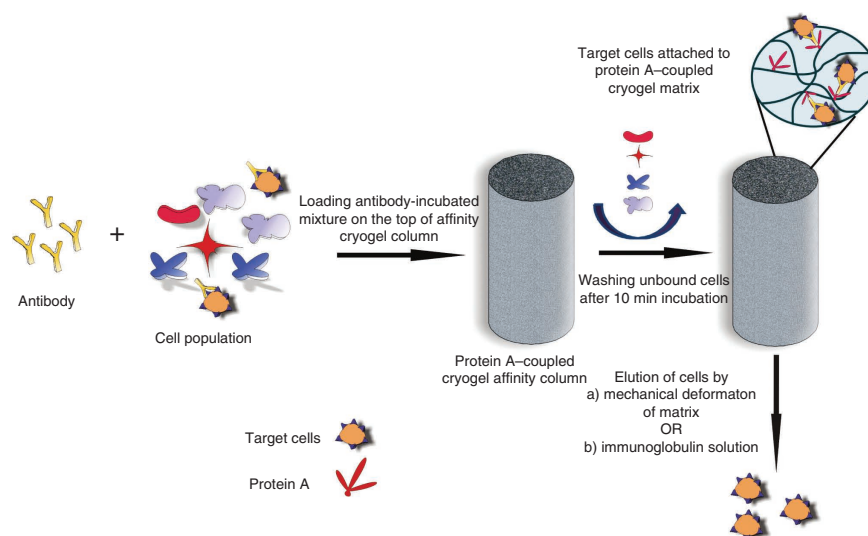
### Cell separations using supermacroporous cryogels

The protocol describes the separation of mammalian cells using a supermacroporous monolithic cryogel column. Cryogel monoliths were prepared both in the column format<sup>20</sup> and in 96-minicolumn plate format<sup>21</sup> for their application in cell chromatography. The pore diameter of up to 100  $\mu$ m (and even larger) enables its application for chromatography of cells of varying size (between 1 and 15  $\mu$ m) without being mechanically entrapped in the column. It was shown that a pulse of blood can be convectively transported by liquid flow through the monolithic plain cryogel column without any physical entrapment of blood cells (Fig. 2). Convectively transported cells do not experience large shear forces because of laminar flow of liquid in the interconnected pores in the monolithic cryogel. Cells can bind to the monolithic cryogel column through specific interactions with some immobilized groups (charges, hydrophobic moieties or affinity ligands) introduced on the surface of their pore walls. For example, *Escherichia coli* cells were captured on an ion-exchange monolithic cryogel column at low ionic strength and were then eluted with 70–80% recovery at 0.4 M NaCl concentration. The same cells can also bind to a Cu(II)-coupled cryogel column and have been recovered using either 100 mM imidazole or 20 mM EDTA<sup>22</sup>. Moreover, different microbial cells having different cell-surface properties can be specifically separated from the mixed population. This was demonstrated on the mixtures of wild-type *E. coli* and recombinant His-tagged *E. coli* cells as well as wild-type



**Figure 2** | Human blood is passed through a cryogel column showing the convective movement of large particles such as mammalian cells.

**Figure 3** | The cell separation strategy used for stem cell separation from umbilical cord blood. Monoclonal antibody was incubated with cell population to label a specific cell type. The cell population containing the labeled target cells was then passed through a protein A-coupled epoxy-activated cryogel column. After 10 min of incubation, the affinity column was washed with buffer to remove unbound cells. The target cells bound on the affinity cryogel column were then eluted by mechanical squeezing of the affinity cryogel matrix as shown in **Figure 4**.



*E. coli* and *Bacillus halodurans* cells<sup>23</sup>. This approach was also used for the separation of yeast and *E. coli* cells on immobilized concanavalin A as ligand on the cryogel monolith<sup>24</sup>. The cryogel matrices were also applied for the separation of a variety of bioparticles, such as inclusion bodies<sup>25</sup>, mitochondria<sup>26</sup> and viruses<sup>27</sup>.

Cell affinity chromatography using supermacroporous monolithic cryogels proved to be an efficient approach for the separation of specific cell types such as T and B lymphocytes<sup>20,28</sup>. Antibody-based affinity interactions are remarkable and highly efficient as ligands for cell separation because of their great diversity and specificity. The interaction of antibody with a specific cell-surface antigen is generally used for isolation of a particular cell type. The concept of affinity separation follows either negative selection or positive selection of cells. In negative selection, nonspecific cells attach to the affinity ligand on the support matrix and the target cells pass through the matrix unretained. In contrast, positive selection involves the specific binding of target cells to the affinity adsorbent; after washing, bound cells can be recovered from the matrix in a purified form.

However, the cell affinity chromatographic method using cryogels has been used here in a more generic manner. A cryogel affinity matrix has been developed by covalent coupling of protein A on the surface of the pore wall, which can capture IgG-labeled specific cells. This provides an advantage over the existing methods of cell separation such as magnetic affinity cell sorting and flow cytometry. Protein A consists of a 40- to 60-kD protein present in the cell wall of *Staphylococcus aureus* that has affinity to the Fc portion of the IgG from a wide range of species. Protein A can be covalently coupled to epoxy-activated cryogel monolith by two-step derivatization using functionalization of epoxy-activated cryogel with ethylenediamine and then crosslinking of protein A using glutaraldehyde. The protein A-bound monolithic cryogel can be used as a generic affinity adsorbent for specific antibody (IgG type)-labeled cells and can thus separate them from nonspecific cells in the mixture. After treating cells with antibodies (IgG type) to the cell-surface receptors, the cell mixture was loaded onto the cryogel-protein A column. The antibody-labeled cells bound to protein A through the Fc region of the labeled antibody, whereas nonlabeled cells remained unbound and passed through the column (**Fig. 3**). The results were compared with control experiments in which lymphocytes and umbilical cord blood (UCB) without antibody labeling were passed through both protein A-coupled and

protein A noncoupled cryogel columns. The protocol is detailed in the PROCEDURE. With this separation strategy, the generic protocol presented here describes the separation of mammalian cell types, with the particular examples of fractionation of T and B lymphocytes from human blood buffy coat (Step 16A(i-viii) of PROCEDURE) and hematopoietic stem cells (CD34<sup>+</sup>) from human UCB (Step 16B(i-ix) of PROCEDURE). The established cell separation approach using affinity cryogels can be integrated in many cell-based studies and for cell-based therapy in which a large purified cell population is required.

#### Experimental design: cell separation strategy

**Affinity cryogel matrix.** Cryogel matrices were developed in monolithic format to provide a suitable chromatography matrix for cell separation. Two different matrices were developed on the basis of acrylamide (AAM) derivatives such as AAM and/or *N,N*-dimethyl acrylamide (DMAAM). Both of the cryogels developed can be used as cell separation matrix. The epoxy group was incorporated by copolymerization with allylglycidyl ether (AGE). The final optimum concentration of monomers can vary from 5% to 6% (wt/vol). Above this concentration of monomers, the optimum pore size for convective movement of cells in the cryogel decreases; below this concentration, the cryogel formed does not have enough mechanical strength and does not retain its shape. The freezing temperature is very critical for cryogel synthesis. Here, the cryogel was prepared at  $-12^{\circ}\text{C}$  to provide a sufficiently large pore size. However, the cryogel can be prepared at a temperature range of  $-10$  to  $-15^{\circ}\text{C}$ . Below  $-15^{\circ}\text{C}$ , the pore size of the cryogel decreases and above  $-10^{\circ}\text{C}$  the developed gels become loose with low porosity. The cryogel matrix was covalently coupled with protein A (ligand) to capture different cell types labeled with a specific IgG antibody. Scanning electron microscopy images of the prepared cryogel should be taken to check the pore size of the synthesized column. The pore size should be 50–100  $\mu\text{m}$ . The water flow parameter should also be checked for its applicability to chromatography. The volumetric flow rate of the aqueous solvent should be around 5–8  $\text{ml min}^{-1}$ . The synthesized cryogel column should be properly packed in a syringe without any leakage (refer to TROUBLESHOOTING table).

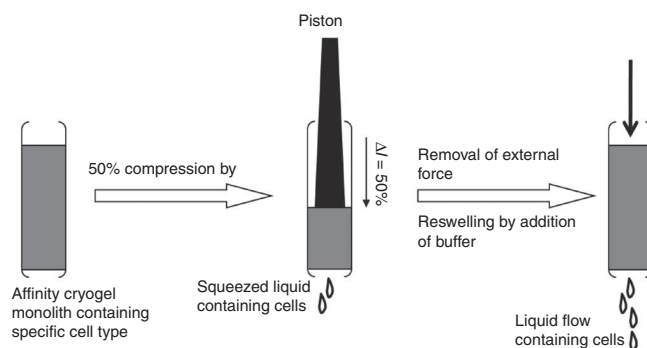
## PROTOCOL

**Cell binding.** All the cell chromatography experiments were conducted under sterile conditions and inside a cell culture biosafety laminar hood. Cells were incubated with a specific antibody, e.g., goat anti-human IgG and anti-human CD34<sup>+</sup>. Labeled cells were then applied to a protein A–coupled cryogel column. Here, the cells were labeled before chromatography; this approach enhances the binding capacity of cells. Labeling the matrix with an antibody before chromatography can cause disorientation of the bound antibody, which does not provide an accessible antigen-binding fragment ( $F_{ab}$ ) for efficient cell binding. The cell binding on the protein A–monolithic cryogel column was carried out as presented schematically in **Figure 3**. As a control experiment, lymphocytes and UCB without antibody labeling were passed through both protein A–coupled cryogel column and cryogel column without protein A. The detailed protocol is presented in the PROCEDURE.

**Cell recovery.** Target cells were recovered by two different approaches:

- Treatment with immunoglobulin (Ig) solution: In this approach, cells can be specifically eluted. The fresh solution of secondary antibody was passed through the affinity cryogel matrix bound with targeted cells. This step causes desorption of bound cells because of its displacement with a higher-affinity secondary antibody.
- Mechanical deformation of cryogel matrix: The recovery of captured cells was carried out by the compression of an affinity cryogel matrix to ~50% of its original length and the collection of squeezed liquid. Thereafter, the compression force was released and the compressed affinity matrix was reswollen with buffer to regain its original size and shape, followed by immediate flow of buffer (10 ml) at a flow rate of 2 ml min<sup>-1</sup> (**Fig. 4**).

The developed protocol for cell separation will remain the same for all cell types. The step that might need optimization for some cell types is the one that involves preclarification of unwanted



**Figure 4** | Mechanical squeezing of the cryogel matrix for the recovery of bound cells. Affinity-bound cells on cryogel matrix were removed by compressing the cryogel column to up to 50% of its original length by using an external piston that squeezes out the liquid that contains cells. In the next step, the buffer was added to reswell the affinity cryogel matrix and then the column was flushed with fresh cold buffer.

cells from the mixture to increase the binding and recovery of cells after binding (see **Box 1**). As proposed in the above-mentioned cell recovery process, cells can be recovered by 50% compression of the cryogel column with a subsequent buffer wash. However, for the separation of other cell types, it is possible that compression percentages could be more (~80%) or a little less (~30%) than what is proposed in PROCEDURE Step 16A(viii) and B(ix).

An unlabeled sample mixture of cells is an appropriate control to run in parallel to the chromatography of cells. It should pass through the protein A–coupled cryogel column, and retention of these unlabeled cells should be compared with that of the labeled sample. The obvious result of the control experiment is that there should not be any nonspecific binding of cells in the cryogel column. In the present protocol, as a control experiment, lymphocytes without antibody labeling were passed through both a protein A–cryogel column and a cryogel column without protein A.

## BOX 1 | PREPARATION AND CONCENTRATION OF MONONUCLEAR CELLS OR LYMPHOCYTES

1. Add Ficoll-Paque PLUS (3 ml) to the centrifuge tube.
2. Carefully add a layer of diluted blood sample (4 ml) over Ficoll-Paque PLUS.
3. Centrifuge at 400g for 30–40 min at 18–20 °C.
4. Pipet out the upper layer using a clean Pasteur pipette, leaving the lymphocyte layer undisturbed at the interface. Do not disturb the lymphocyte layer.
5. Now draw off the lymphocyte layer to a clean centrifuge tube by using a clean Pasteur pipette. It is critical to remove all the material at the interface but in a minimum volume.
- ! **CAUTION** Removing excess Ficoll-Paque PLUS causes granulocyte contamination; removing excess supernatant results in platelet contamination.
6. Add at least three volumes (6 ml total) of balanced salt solution to the lymphocyte-concentrated sample in the test tube and gently suspend cells using a Pasteur pipette.
7. Centrifuge at 60–100g for 10 min at 18–20 °C.
8. Discard the supernatant.
9. Suspend cells in 6–8 ml of balanced salt solution by gently drawing them in and out using a Pasteur pipette.
10. Repeat Steps 7–8, and the lymphocyte-concentrated cell suspension should now be suspended in the medium appropriate to the application.

▲ **CRITICAL STEP** To obtain a sufficiently pure preparation of lymphocytes, recentrifugation with Ficoll-Paque can be necessary to remove contaminating red blood cells. When layering the sample, do not mix the Ficoll-Paque PLUS and the diluted blood sample.



## MATERIALS

### REAGENTS

- Acrylamide (AAM, ≥99% (wt/wt); Sigma-Aldrich, cat. no. A9099, CAS no. 79-06-1) **! CAUTION** It is an irritant, and is very toxic if inhaled, if it comes in contact with skin, and if swallowed.
- *N,N'*-Dimehtylacrylamide (DMAAM, 99%; Sigma-Aldrich, cat. no. 274135, CAS no. 2680—03-7) **! CAUTION** It is toxic if inhaled; use eyeshields, faceshields, full-face respirator, and gloves while handling.
- *N,N'*-methylenebisacrylamide (MBAAM, ≥98%; Sigma-Aldrich, cat. no. M7279, CAS no. 110-26-9) **! CAUTION** It is an irritant, and is very toxic if inhaled, if it comes in contact with skin, and if swallowed.
- Allylglycidyl ether (AGE, >99%; Sigma-Aldrich, cat. no. A32608, CAS no. 106-92-3) **! CAUTION** It is an irritant and a moderate skin and severe eye irritant on contact. Use protective gloves, eye and face shields.
- Ammonium persulfate (APS; Bio-Rad Labs, cat. no. 161-0700, CAS no. 7727-54-0) **! CAUTION** It is an irritant and may cause fire on contact with flammable materials. Use protective gloves and glasses.
- *N,N,N',N'*-tetramethylethylenediamine (TEMED, Bio-Rad Labs, cat. no. 161-0801, CAS no. 110-18-9) **! CAUTION** It is highly flammable and causes burns. Use protective gloves and glasses. Keep away from heat and open flame.
- Glutaraldehyde solution (grade 1, 50% in water; Sigma-Aldrich, cat. no. G7651, CAS no. 111-30-8) **! CAUTION** It is highly flammable, and is toxic if inhaled, if it comes in contact with skin and if swallowed. Keep the container tightly closed and in a cool place.
- Ethylenediamine (99%; Sigma-Aldrich, cat. no. E26266, CAS no. 107-15-3) **! CAUTION** It is corrosive and flammable. It is harmful on contact with skin and if swallowed. It causes burns.
- Ethylenediaminetetraacetic acid (EDTA, tetrasodium salt dihydrate >99.5%, 50 mM solution; Sigma, cat. no. E6511, CAS no. 10378-23-1) **! CAUTION** While handling all these chemicals and reagents, use protective gloves and glasses and work in a fume hood.
- Goat anti-human IgG (Zymed Laboratories)
- Anti-human CD34<sup>+</sup> was produced in ascites by injecting HSCA hybridoma cells (Kaneka Chemical Industries) into mice, and purified using a protein G column (1 ml; PerSeptive Biosystems)
- Protein A (Bangalore Genei, cat. no. 105254). **▲ CRITICAL** Store below 0 °C.
- Ficol-Paque PLUS (GE Life Sciences)
- Penicillin-streptomycin solution (Sigma-Aldrich, 10,000 units penicillin and 10 mg ml<sup>-1</sup> streptomycin, cat. no. P4333)
- Hydroxyethyl cellulose, high viscosity (HEC; LOBA Chemie, cat. no. 4137, CAS no. 9004-62-0)
- Sodium citrate (Merck, cat. no. 567446, CAS no. 6132-04-3)
- Citric acid, anhydrous (Merck, cat. no. 61804105001730)
- Dextrose or glucose (Merck, cat. no. 346351, CAS no. 50-99-7)
- Sodium borohydride (NaBH<sub>4</sub>; Merck, cat. no. 1063710100, CAS no. 16940-66-2)
- Tris buffer (Merck, cat. no. 61771401001730)
- Hydrochloric acid, 35% pure (HCl; Merck, cat. no. 61752690251730)
- Sodium azide (Merck, cat. no. 61824401001730)
- Ethanol (Merck, cat. no. 1085430250, CAS no. 64-17-5) **! CAUTION** Highly flammable liquid and vapor. Keep away from heat/sparks/open flames/hot surfaces.
- Calcium chloride (CaCl<sub>2</sub>; Merck, cat. no. 1023780500, CAS no. 10043-52-4) **! CAUTION** Causes serious eye irritation. In case of contact with eyes, rinse with water for several minutes.
- Magnesium chloride (MgCl<sub>2</sub>; Merck, cat. no. 8147330100, CAS no. 7786-30-3)
- Potassium chloride (KCl; Merck, cat. no. 61753325001730)
- Sodium chloride (NaCl; Merck, cat. no. 61751990201730)
- Sodium phosphate monobasic (NaH<sub>2</sub>PO<sub>4</sub>; Merck, cat. no. 567545, CAS no. 10049-21-5)
- Sodium phosphate dibasic (Disodium hydrogen phosphate) (Na<sub>2</sub>HPO<sub>4</sub>; Merck, cat. no. 567550, CAS no. 7558-79-4)
- Distilled water
- Nitrogen (N<sub>2</sub>) gas
- Bovine serum albumin (BSA; Merck, cat. no. 12657, CAS no. 9048-46-8) **▲ CRITICAL** store at 2–8 °C.
- Sodium chloride (NaCl; Merck, cat. no. 567440, CAS no. 7647-14-5)
- Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>; Merck, cat. no. 60487305001730)

- Hydrochloric acid (HCl; Merck, cat. no. 60906005001730)
- Sodium hydroxide (NaOH; Merck, cat. no. 61757390511730)
- Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>; Merck, cat. no. 61784490511730)
- Sodium bicarbonate (NaHCO<sub>3</sub>; Merck, cat. no. 61752090501730)
- Sodium acetate trihydrate (Merck, cat. no. 61751890201730)
- HEPES (Merck, cat. no. 1152310025, CAS no. 75277-39-3)
- Acetic acid, glacial (Merck, cat. no. 61780790251730)

### EQUIPMENT

- Thermostatic bath/circulator (Proline 1840; Lauda-Wobser, cat. no. LCK 1891)
- Laminar hood
- Florescent activated cell scanner (Cyflow; Partec, cat. no. CY-S-3001)
- Sterile tubes for blood collection (50 ml; Tarsons Products, cat. no. 546040)
- Conical flasks
- Filter (0.2-µm pore size)
- Vacuum pump (Rocker 300; Tarsons Products, Rocker 300, cat. no. 7010)
- Magnetic stirrer and stir bars (Tarsons Products, cat. no. 4020)
- Plastic syringes (any company with required size range of 2 ml to 20 ml)
- Peristaltic pump (P1; GE Healthcare, cat. no. 18-1110-91)
- Centrifuge (Hermle Labortechnik Model Z323)
- Pasteur pipette
- Sterile vials (for collecting bound cells from compressed cryogel columns, 2 ml) (Tarsons Products, cat. no. 500020)
- Polystyrene pipe (Tygon vacuum tubing; Tarsons Products, cat. no. 740000)

### REAGENT SETUP

**Phosphate buffer (0.01 M), pH 7.4** Add 57 ml of 0.02 M sodium phosphate monobasic and 243 ml of 0.02 M sodium phosphate dibasic heptahydrate. Dilute the mixture with distilled water to bring the total volume up to 600 ml.

**Phosphate buffer (10 mM) containing 0.15 M NaCl (pH 7.4)** Recipe is the same as that for the preparation of phosphate buffer (0.01 M) pH 7.4, with the exception of the addition of 0.15 M NaCl.

**Phosphate-buffered saline (PBS; pH 7.2) supplemented with 0.5% bovine serum albumin (BSA)** For 1 liter PBS, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na<sub>2</sub>HPO<sub>4</sub> and 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 800 ml distilled water. Then adjust the pH to 7.4 with HCl and NaOH and add distilled water to bring the volume to 1 liter. Add 0.5 % (wt/vol) bovine serum albumin.

**Sodium carbonate buffer (pH 9.2)** Mix 10 ml of 0.1M Na<sub>2</sub>CO<sub>3</sub> and 90 ml of 0.1M NaHCO<sub>3</sub>

**HEPES buffer (pH 7.4)** Mix 20 mM HEPES in 100 ml distilled water and then add 0.2 M NaCl.

**Acetate buffer (pH 2.5)** Mix 10 ml of 0.2 M sodium acetate trihydrate and 90 ml 0.2 M acetic acid.

**Balanced salt solution** Combine 0.145 M Tris-HCl (pH 7.6) containing 0.1% glucose, 0.05 mM CaCl<sub>2</sub>, 0.98 mM MgCl<sub>2</sub>, 5.4 mM KCl and 14 mM NaCl.

**Acid Citrate Dextrose (ACD) or sodium citrate (anticoagulant)** This can be prepared by performing the following steps:

- Dissolve 1.32 g of sodium citrate in 85 ml of distilled water
- Dissolve 0.48 g of citric acid in the solution from step 1
- Dissolve 1.47 g of dextrose in the solution from step 2
- Add distilled water and make up to 100 ml
- Filter-sterilize through a 0.2-µm-pore filter
- Use 0.25 ml of solution for 1 ml of blood

**Umbilical cord blood** Under aseptic conditions inside a laminar hood, collect UCB into a sterile tube containing anticoagulant (ACD or sodium citrate) and store at 4 °C. It is better to carry out experiments with fresh blood (within 6 h after collection) to avoid contamination and to capture the CD34<sup>+</sup> cells. Delaying the process may lose CD34<sup>+</sup> expression.

**Peripheral blood** Under sterile conditions, collect whole blood in a sterile tube containing anticoagulant (heparin or ACD) and store at 4 °C. It is better to carry out the experiment with fresh blood (within 6 h after collection) to avoid contamination and to capture CD34<sup>+</sup> cells. Delaying the process may lose CD34<sup>+</sup> expression. **▲ CRITICAL** Appropriate ethical approval should be taken before the collection of UCB and peripheral blood for stem cell isolation. Consent from parents should also be taken before the collection of UCB after childbirth.

## PROCEDURE

### Preparation of supermacroporous monolithic cryogel–protein A affinity matrix

1| To degas distilled water, fill the distilled water to half the volume in a conical flask provided with a nozzle on its neck. Attach one end of a polystyrene pipe to the nozzle and the other end to a vacuum pump to create a vacuum in the conical flask. During vacuum, the water should be under constant magnetic stirring. Allow the degassing to continue for ~1 h.

2| Choose which polymer is the most appropriate for your experiment. Two different types of AAm-based cryogels can be prepared: (A) DMAAm-based cryogel and (B) AAm-based cryogel.

▲ **CRITICAL STEP** Both AAm and DMAAm are suitable polymers for the preparation of cryogel-based chromatography matrix. Highly hydrophilic matrices are preferred for chromatography purposes to avoid nonspecific interactions during the separation process. Both the matrices are hydrophilic in nature and are appropriate for cryogel preparation. AAm is slightly more hydrophilic than DMAAm and is a preferred choice of polymer for cryogel preparation. In contrast, DMAAm can be stored more stably than AAm. However, the overall porosity, pore size and mechanical strength of the developed cryogel matrix are approximately similar and they have the same physical morphology.

#### (A) DMAAm-based cryogel

(i) In 100 ml of degassed water, mix monomers, 6.3 ml of DMAAm and 0.85 ml of AGE.

(ii) Dissolve 2.2 g of MBAAm as a crosslinker in the monomer solution. A total concentration of 5% monomeric precursors should be used for the cryogel formation.

#### (B) AAm-based cryogel

(i) Mix monomers, 4 g of AAm and 1 ml of AGE, in 100 ml of degassed water.

(ii) Dissolve 1 g of MBAAm as a crosslinking agent in monomer solution. A total concentration of 6% monomeric precursors should be used for the cryogel formation.

3| Next, keep the mixture under constant N<sub>2</sub> purging for 5–10 min to remove dissolved oxygen.

4| Cool the mixture at 4 °C and then add 110 mg of APS, followed by 95 µl of TEMED as initiator/activator agent.

▲ **CRITICAL STEP** Ensure that the water is properly degassed and purged with N<sub>2</sub> before the addition of APS/TEMED to remove O<sub>2</sub> (to inhibit free-radical polymerization).

▲ **CRITICAL STEP** The mixture of monomers should be cooled to 1–4 °C before the addition of APS/TEMED to retard the free-radical reaction.

5| After addition of APS/TEMED, quickly pour 1 ml of the mixture into precooled plastic syringes (diameter 0.9 cm) and store at –12 °C in a refrigerated circulatory bath for 12 h.

To process large sample volumes (up to 50 ml), we recommend an optimal column size of 20 ml (length 6.3 cm × diameter 2 cm). The column can be prepared as per the sample volume and scaling up of chromatography. To prepare such a column, pour the desired volume of the monomer mixture into defined plastic syringes (e.g., 5 ml mixture into a 5 ml syringe, 10 ml mixture into a 10 ml syringe and so on). However, it is not recommended to increase the column size beyond 20 ml, as it may not be very uniform.

6| After a complete incubation period of 12 h, thaw the synthesized cryogel with ~20 ml of water at room temperature (25 °C), followed by washing with another portion of 20 ml of water and water/ethanol mixture to remove any unreacted monomers.

7| After complete washing, dry the cryogel monolith in a vacuum and store it for further use.

■ **PAUSE POINT** The cryogels in dried form can be stored from days to weeks or even for several months for further modification and use. However, in the wet form, the gel needs to be modified soon after the cryogel washing in Step 5.

## ? TROUBLESHOOTING

### Two-step derivatization of epoxy-containing cryogel monolith for the attachment of protein: reaction with ethylenediamine

8| Place 1-ml cryogel columns (dried or freshly prepared wet) in a 50-ml plastic tube closed with a lid. One to five gel columns can be placed in a single tube.

9| Wash cryogel columns with water (10 ml per gel) and then with 0.2 M Na<sub>2</sub>CO<sub>3</sub> (pH >10; 10 ml per gel).

10| Incubate cryogel columns with ethylenediamine (0.5 M in 0.2 M Na<sub>2</sub>CO<sub>3</sub>; 10 ml per gel) for 4 h and wash with water until the pH is close to neutral.

▲ **CRITICAL STEP** Reactions and water washes are carried out by gently shaking the gels in the tube on a pedal shaker as indicated or for a minimum of 5 min.

## Reaction with glutaraldehyde

- 11|** Wash the ethylenediamine-treated cryogel columns with 0.1 M sodium phosphate buffer (pH 7.2; 10 ml per gel).
- 12|** Next, incubate the cryogel columns in glutaraldehyde solution (5% (vol/vol); 10 ml per gel) in 0.1 M sodium phosphate buffer (pH 7.2; for 5 h).
- 13|** Wash the columns in distilled water (10 ml per gel, washing repeatedly three to five times).
- 14|** Incubate the derivatized cryogel matrix with the solution of protein A ( $1.6 \text{ mg ml}^{-1}$ ; 10 ml) in 0.1 M sodium phosphate buffer (pH 7.2) at 4 °C for 24 h. The unreacted protein A solution after this step can be reused if needed.
- 15|** Finally, wash the protein A-coupled cryogel matrix with freshly prepared sodium borohydride ( $\text{NaBH}_4$ ) solution (0.1 M in sodium carbonate buffer (pH 9.2); 10 ml per gel) to reduce Schiff's base formed between the protein and the aldehyde-containing matrix.
- PAUSE POINT** The protein A-coupled column can be stored in phosphate buffer (0.1 M, pH 7.2) at 4 °C for several months in the presence of 0.02% (wt/vol) sodium azide or 20% (vol/vol) ethanol.
- 16|** The column can now be used for cell separations. To illustrate how this is done, we have included procedures for two examples: (A) fractionation of lymphocytes and (B) separation of  $\text{CD34}^+$  stem cells from UCB.

### (A) Fractionation of lymphocytes

- (i) Separate mononuclear cells (lymphocytes, buffy coat) from freshly collected human peripheral blood using Ficoll-Paque PLUS density gradient separation medium by centrifugation at 400g for 40 min at room temperature as per the manufacturer's instructions. The detailed procedure as described in a handbook available from GE Life Sciences<sup>29</sup> can be found in **Box 1**. As a control experiment, lymphocytes without antibody labeling were passed through both protein A-coupled cryogel column and cryogel column without protein A.
- (ii) Place 2 ml of cryogel column into a sterile plastic syringe and wash thoroughly by passing 20 ml of 20 mM HEPES buffer (pH 7.4) containing 0.2 M NaCl through it at a flow rate of  $2 \text{ ml min}^{-1}$  using a peristaltic pump. (This process can be scaled up. The column size can be increased to process a 50 ml sample. The optimum column size of 20 ml (length 6.3 cm  $\times$  diameter 2 cm) can be used to process a 50 ml sample volume in recycle mode).
- ? TROUBLESHOOTING**
- (iii) Next, add a small amount ( $<0.3 \text{ ml}$ ) of buffer above the top surface of the cryogel column.
- (iv) Treat the lymphocytes ( $1 \text{ ml}$ ,  $2.0\text{--}4.0 \times 10^7$  cells per ml) with  $50 \mu\text{l}$  ( $0.1 \mu\text{g } \mu\text{l}^{-1}$ ) of goat anti-human IgG and incubate at 4 °C for 5 min. This will bind to B lymphocytes having surface IgG molecules.
- (v) Centrifuge cells at 200g for 5 min and suspend the cells in 1 ml of balanced salt solution.
- (vi) Add antibody-treated lymphocytes to the top of the column and collect 1.5 ml (about void volume of the column) flow-through. Close the column outlet and allow the cells to bind efficiently to the matrix for 10 min without any buffer flow, at room temperature.
- (vii) After 10 min of incubation, run the cold HEPES buffer (20 ml) through the column at a flow rate of  $1.5 \text{ ml min}^{-1}$  to wash out unbound or loosely bound cells.

#### ? TROUBLESHOOTING

- (viii) Bound cells can be recovered by either of two ways. One approach is to recover cells with Ig solution after complete washing. To do this, first incubate the column and the buffer at 37 °C for 10 min and then add 2 ml of IgG ( $30 \text{ mg ml}^{-1}$ ) through the column. Thereafter, incubate the column at room temperature without buffer flow for 5 min. Next, run 20 ml of buffer through the column at a flow rate of  $1.5 \text{ ml min}^{-1}$  and collect a 4-ml aliquot containing a specific cell type. An alternative approach is to compress the cryogel column gently with a syringe piston to up to 50% of its original length and collect the squeezed liquid in a sterile vial. Thereafter, flow the buffer (10 ml) at a flow rate of  $1.5 \text{ ml min}^{-1}$  to recover the remaining loosely bound cells on the cryogel column. The process described here is used for the mechanical squeezing of cryogel for the recovery of bound cells.

#### ? TROUBLESHOOTING

### (B) Stem cell ( $\text{CD34}^+$ ) separation from UCB

- (i) Sediment red blood cells (RBCs) from UCB by using hydroxyethyl cellulose (HEC) as the sedimenting agent. Add 6% (vol/vol) HEC into the blood at a 5:1 ratio of blood:HEC, and mix properly. Thereafter, allow RBCs to settle down for 15–20 min, then remove the settled RBCs and store supernatants containing mononuclear cells.
- ▲ CRITICAL STEP** To achieve high purification and high yield, preclarification is a desired step. The RBC and/or platelets can be removed from whole blood by using sedimenting agents, e.g., Ficoll-Paque PLUS and HEC, as discussed in step **Box 1** and PROCEDURE Step 16B(i), respectively.
- ? TROUBLESHOOTING**
- (ii) Incubate the RBC-sedimented UCB (1 ml) and whole UCB (1 ml) with human anti- $\text{CD34}$  ( $1 \mu\text{g ml}^{-1}$ ) at 4 °C for 5 min.
- (iii) Centrifuge the cells at 200g for 5 min and suspend in 1 ml of PBS (pH 7.2, supplemented with 0.5% BSA).

## PROTOCOL

- (iv) Sterilize the protein A-coupled cryogel column (1 ml; height × diameter: 1.0 cm × 0.9 cm) by passing 20 ml of 2% (vol/vol) penicillin-streptomycin solution in buffer (0.1 M PBS, pH 7.4) at a flow rate of 1.5 ml min<sup>-1</sup>.
- (v) Thereafter, equilibrate the sterilized protein A-cryogel matrix by passing 50 ml of PBS (pH 7.2 supplemented with 0.5% BSA) at a flow rate of 1.5 ml min<sup>-1</sup>.
- (vi) Close the lower end of the cryogel column and leave <0.2 ml buffer above the top surface of the column.
- (vii) Load 0.8 ml of antibody-treated, UCB and RBC-sedimented onto the top of the cryogel column. Thereafter, open the lower end of the cryogel column and collect 0.6 ml of flow-through. Close the column outlet and allow the cells to bind efficiently to the matrix for 10 min without any buffer flow at room temperature.
- (viii) After 10 min of incubation time for cell binding, wash the column with 10 ml of buffer at a flow rate of 1.5 ml min<sup>-1</sup> to remove unbound or loosely bound cells.
- (ix) Bound cells can be recovered by either of two ways. One approach is to recover cells with Ig solution after complete washing. To do this, first, incubate the column and the buffer at 37 °C for 10 min and then add 1 ml of IgG (30 mg ml<sup>-1</sup>) through the column. Thereafter, incubate the column at room temperature without buffer flow for 5 min. Now, run 10 ml of buffer through the column at a flow rate of 1.5 ml min<sup>-1</sup> and collect 3 ml of aliquot containing a specific cell type. An alternative approach is to compress the cryogel column gently with the syringe piston to up to 50% of its original length and collect the squeezed liquid in a sterile vial. Thereafter, run the buffer (10 ml) at a flow rate of 1.5 ml min<sup>-1</sup> to recover the remaining loosely bound cells on the cryogel column. The process described here is used for the mechanical squeezing of cryogel for the recovery of bound cells.

**▲ CRITICAL STEP** If the recovery of cells by mechanical squeezing is <70% in the first step, we suggest repeating it.  
**? TROUBLESHOOTING**

### Regeneration of the column

**17|** Used columns (1 or 2 ml) can be regenerated by passing 10 column volumes of 50 mM acetate buffer (pH 2.5) at a flow rate of 1 ml min<sup>-1</sup>.

**18|** Equilibrate the column by passing 20 column volumes of 0.1 M PBS (pH 7.2).

### ? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

**TABLE 1 |** Troubleshooting table.

Step	Problem	Solution
7	After synthesis, some of the cryogels are irregular in shape	Choose only those synthesized cryogels with proper shape as shown in <b>Figure 5</b>
16A(ii)	Sample or liquid leaks from the sides of the cryogel matrix packed in the syringe	Use only those cryogels that are tightly packed in syringes. Incubate the packed cryogels with water for complete swelling. Check each cryogel's packing by inverting the packed syringe to see whether the cryogel matrix moves or not. If the cryogel slides, the matrix is not properly packed. Either change the matrix or swell the matrix for additional time
16A(vii)	The target cells are eluted out of the column during the washing procedure	Decrease the flow rate of washing buffer. Check the pH of washing buffer Check for leakage of cells through the sides of the cryogel matrix in the syringe or appropriate mold (refer to TROUBLESHOOTING advice for Step 16A(ii))
16A(viii)	Low recovery of bound cells	Repeat the cell release process two or three times
16A(viii), 16B(ix)	Bound cells do not release after mechanical squeezing of the cryogel	Compress the cryogel again with 70–80% compression followed by liquid flow at 1.5 ml min <sup>-1</sup> . If cells are not coming out even after this treatment, then the developed matrix does not have sufficient pore size. Please check the pore size of developed cryogel before chromatography
	Released cells are not viable	Carry out the viability test before and after the chromatography Decrease the compression on cryogel, restrict the compression up to 25% of original length, change in length followed by buffer flow (1 ml min <sup>-1</sup> ) Maintain the temperature during chromatography; it should be less than room temperature. Pass cold buffer through the column to wash the column and to elute unbound cells The complete chromatography process should not take more than 30 min
16B(i)	Very low recovery percentage of CD34 <sup>+</sup> cells	Process the UCB within 2 h after collection or refer to TROUBLESHOOTING advice for Step 16A(vii), 16A(viii) and 16B(ix)



## TIMING

Steps 1–7: ~17 h  
Steps 8–16: 40–48 h  
Step 16A(i): ~1 h  
Step 16A(ii–viii): 45–60 min  
Step 16B(i): ~30 min  
Step 16B(ii–ix): ~60 min  
Steps 17 and 18: 30 min

## ANTICIPATED RESULTS

### Affinity cryogel matrix design for cell chromatography

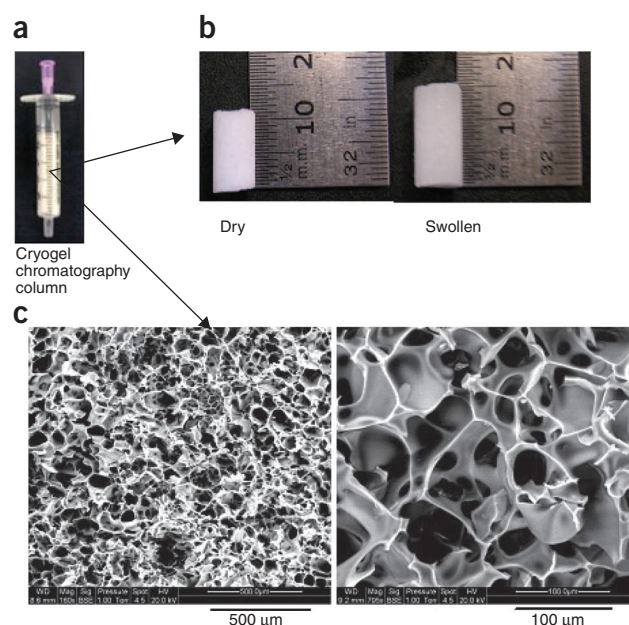
The cell chromatography matrix was synthesized by copolymerization of AAm and AGE using MBAAm as crosslinker at  $-12^{\circ}\text{C}$  for 12 h. The water-swollen poly(acrylamide-co-allylglycidyl ether) (poly(AAm-co-AGE)) cryogel maintained its shape and morphology even after complete drying (Fig. 5a,b). Water-swollen poly(AAm-co-AGE) cryogels were spongy in nature and water could be squeezed out with compression. Analysis by scanning electron microscopy demonstrated that the pore diameter of dried poly(AAm-co-AGE) cryogels is in the range of 50–100  $\mu\text{m}$  (Fig. 5c). The thick and smooth sidewalls of pores were observed by scanning electron microscopy (Fig. 5c). These large interconnected pores in suitably designed cryogel matrix can function as capillaries and can quickly absorb a large amount of water. These swelling studies indicated that these matrices have a high rate of swelling. The flow properties were checked for protein A-coupled poly(AAm-co-AGE) cryogel matrix and showed a high volumetric flow rate of around  $8\text{ ml min}^{-1}$  across the cryogel matrix. It was also observed that the cryogel matrix allowed convective movement of large particles. The amount of protein A coupled to the cryogel matrix was found to be ~2 mg per gel matrix, as measured by bicinconnic acid reagent.

### Fractionation of lymphocytes

The interaction of protein A with cells bearing IgG antibodies on their surfaces has been successfully used for the fractionation of blood lymphocytes in a chromatographic format. B lymphocytes are Ig-positive cells and express IgG on the cell surface. The results were calculated by flow cytometry analysis, with the scatter gates set on the lymphocyte fraction. When loaded with 1 ml of treated cells ( $3.0 \times 10^7$  cells per ml), ~91% of B cells were retained in the 2-ml column. With ~11.6% of B cells in the total mononuclear cells, the B-cell-binding capacity was determined to be  $\sim 1.6 \times 10^6$  cells per ml of adsorbent. The cells in the breakthrough fraction were enriched in T cells (81%). The viability of cells investigated in the breakthrough fraction was >90%, as measured by Trypan blue staining of cells. The electron micrograph image (Fig. 6) of bound cells on the protein A-cryogel matrix clearly demonstrated the specific binding of the cells. The cells were attached to the pore walls rather than mechanically entrapped in the dead-flow zones. In all, 70% of the total bound cells were recovered by the treatment of Ig solution, and contain ~70% of B cells (Fig. 7). The overall recovery of B cells from lymphocyte fraction can further be increased if the sample is passed through the column in recycle mode. Moreover, the capacity of the column can be amplified by increasing the size of the affinity column to achieve >90–95% cell recovery. The recovery percentage could be increased by mechanical squeezing of gel. However, the recovered cells also contained ~20% of T cells. Probably, most of the T cells were retained in the column through some weak interactions of cell receptors with protein A or through some cells aggregated with B cells. To achieve higher purification of the recovered B cells, a pulse gradient of human Ig ( $10, 30$  and  $50\text{ mg ml}^{-1}$ ) was applied. At the highest concentration of IgG, 60% B-cell recovery was achieved with <10% T cells. Furthermore, the cells that were directly applied to protein A-cryogel without labeling with goat anti-human IgG were completely recovered by the flow of buffer (Fig. 7). Affinity chromatography of human blood mononuclear cells on supermacroporous cryogel–protein A was found in this study to result in about a twofold enrichment of T cells by negative selection. The very low B-cell contamination (~1%) showed significant selectivity of the separation process. With mechanical squeezing of the cryogel matrix, ~95% of bound cells were recovered, which contained ~70% of B cells. The viability of the recovered cells was ~90%.

### Stem cell chromatography: purification and characterization

**Stem cell (CD34<sup>+</sup>) capture.** Fluorescence-activated cell sorting (FACS) analysis demonstrated that all unwanted cells were washed out during the washing procedure. It was also found that  $\sim 8 \times 10^6$  nucleated cells were removed during washing and



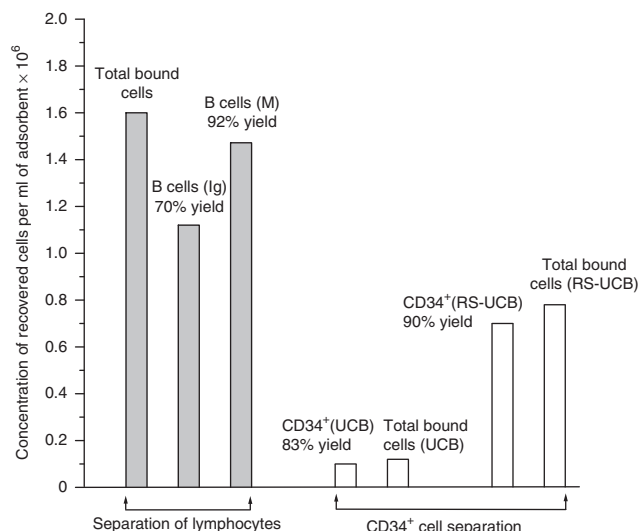
**Figure 5** | Physical characteristics of the monolithic cryogel chromatography column. (a) Cryogel chromatography column. (b) Physical shape of poly(AAm-co-AGE) cryogels in dry and swollen form. (c) Scanning electron microscope images of poly(AAm-co-AGE) cryogels at different magnifications ( $\times 160$  and  $\times 795$ ).



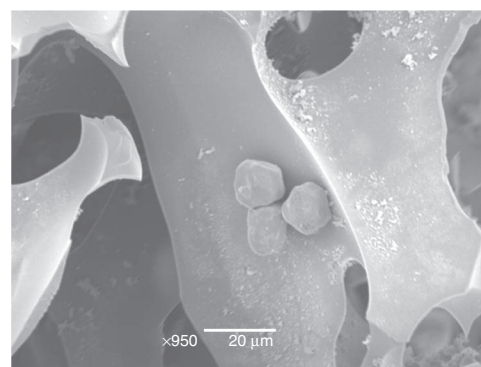
that washed-out nucleated cells may contain some unbound CD34<sup>+</sup> cells. The captured CD34<sup>+</sup> cells were recovered by mechanical squeezing of cryogel. The bound CD34<sup>+</sup> cells were released at 50% compression, followed by 10 ml of buffer wash, to elute loosely bound CD34<sup>+</sup> cells. The FACS analysis showed >95% cell recovery of bound cells (**Fig. 7**). It was also observed that >70% compression of cryogel leads to increase in cell recovery but decrease in viability of eluted CD34<sup>+</sup> cells. Finally, the optimized procedure for cell recovery through mechanical squeezing involves lengthwise compression of up to 50–60%, followed by 10 ml of flushing by PBS. It leads to >95% cell recovery, as confirmed by Trypan blue staining and FACS analysis. The FACS analyses demonstrated that the released or recovered cells were CD34<sup>+</sup>. The pure population of  $1 \times 10^5$  CD34<sup>+</sup> hematopoietic stem cells can be recovered from 0.8 ml of labeled UCB using 1 ml of cryogel matrix. There was an increase in the overall recovery of stem cells from RBC-sedimented UCB, with the recovered cell number of  $7 \times 10^5$  cells per ml of cryogel column. A control experiment was run to demonstrate that  $1 \times 10^6$  nucleated cells can pass through without any nonspecific interactions, and in another experiment, the unhindered movement of whole blood was observed through the cryogel affinity column (**Fig. 7**). There were very few RBCs and platelets that were retained in the column nonspecifically, which affects overall column efficiency as demonstrated in our result in which CD34<sup>+</sup> cells were separated directly from UCB. The overall efficiency of a 1-ml affinity cryogel column was estimated to be around  $7 \times 10^5$  cells per ml of cryogel column (0.9% of total nucleated cells) with >90% purity. The efficiency of the cryogel column decreased to  $1 \times 10^5$  cells per ml of cryogel column (0.2% of total nucleated cells) when applying UCB directly in comparison with RBC-sedimented UCB. We have found 20% CD34<sup>+</sup> cell recovery from UCB and 90% cell recovery from RBC-sedimented UCB using the 1-ml cryogel column (height  $\times$  diameter:  $1.0 \times 0.9$  cm<sup>2</sup>). The capacity of the cryogel column can be increased by changing the physical dimensions to achieve a higher percentage of cell recovery. In another experiment, it was also demonstrated that bound CD34<sup>+</sup> cells can be recovered and concentrated (50% compression followed by 1 ml buffer wash) in 1.5 ml of an elution aliquot containing >90% of bound cells. Overall, the cell chromatographic process took ~30 min.

The isolated cells were grown for 3 weeks and then labeled with FITC-conjugated anti-CD34<sup>+</sup>. The labeled cells were observed under fluorescence microscope; results demonstrated that isolated stem cells maintained proliferation capacity and retained CD34<sup>+</sup> cell-surface markers. We have seen that, during the squeezing process, generally the antibody remains attached to protein A and mostly antibody-free cells can be recovered because the binding strength of protein A to the Fc portion of the IgG antibody is greater than the antibody interaction with the cell-surface markers. We confirmed this when regenerating the protein A–cryogel column with low-pH buffer washing, wherein we observed some concentration of antibody in the column wash. However, it is possible that some of the recovered cells could retain antibody on their surfaces. The antibody bound to recovered cells when kept in growth media can shed the surface-bound antibody during the culture process.

Although we have presented two model examples here for the separation of lymphocytes and CD34<sup>+</sup> cells on a protein A–coupled cryogel column, this approach can be used for other cell types with similar efficiency. We have tried this with other cell types as well. Moreover, the regenerated protein A columns can be reused at least two to three times without showing much effect on the cell binding and recovery.



**Figure 7** | The cell separation efficiency on an affinity-based cryogel chromatographic column (per milliliter adsorbent). Separation of lymphocytes (gray bars) showing B-cell recovery after incubation with immunoglobulin (B cells (Ig)) with 70% yield, and recovery by mechanical squeezing (B cells (M)) with 92% yield from total bound cells on the cryogel adsorbent. CD34<sup>+</sup> cell separation (white bars) showing recovery of CD34<sup>+</sup> cells (CD34<sup>+</sup> (UCB)) with 83% yield directly from UCB and CD34<sup>+</sup> cell recovery (CD34<sup>+</sup> (RS-UCB)) with 90% yield from RBC-sedimented UCB from total CD34<sup>+</sup> cells bound on cryogel adsorbent when direct UCB and RBC-sedimented UCB (RS-UCB) were applied, respectively. In both types of control experiments, i.e., when labeled cells were passed through a plain cryogel column and when unlabeled cells were passed through an affinity cryogel column, no cell binding was observed.



**Figure 6** | Scanning electron micrograph of the inner part of the supermacroporous cryogel-protein A matrix loaded with lymphocytes. The cells are affinity bound on the pore walls. Magnification (x950) (adapted from Kumar *et al.*<sup>20</sup> with permission).

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# Bacterial glycosidases for the production of universal red blood cells

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Enzymatic removal of blood group ABO antigens to develop universal red blood cells (RBCs) was a pioneering vision originally proposed more than 25 years ago. Although the feasibility of this approach was demonstrated in clinical trials for group B RBCs, a major obstacle in translating this technology to clinical practice has been the lack of efficient glycosidase enzymes. Here we report two bacterial glycosidase gene families that provide enzymes capable of efficient removal of A and B antigens at neutral pH with low consumption of recombinant enzymes. The crystal structure of a member of the  $\alpha$ -N-acetylgalactosaminidase family reveals an unusual catalytic mechanism involving NAD<sup>+</sup>. The enzymatic conversion processes we describe hold promise for achieving the goal of producing universal RBCs, which would improve the blood supply while enhancing the safety of clinical transfusions.

The ABO blood group system, discovered in 1900 (ref. 1), is the most important blood group system to consider in transfusion medicine. This system is based on the presence or absence of the blood group antigens A and B. The corresponding blood group carbohydrate structures, designated ABH, are found at the termini of oligosaccharide chains on glycoproteins and glycolipids on the surface of erythrocytes and of endothelial and most epithelial cells<sup>2,3</sup>. The immunodominant monosaccharide that determines blood group A specificity is a terminal  $\alpha$ -1,3-linked N-acetylgalactosamine (GalNAc), whereas the corresponding monosaccharide of blood group B specificity is an  $\alpha$ -1,3-linked galactose (Gal) (Fig. 1). Group O cells lack both of these monosaccharides at the termini of their oligosaccharide chains, which instead are terminated with  $\alpha$ -1,2-linked fucose (Fuc) residues and designated the H antigen (see review<sup>2</sup>). The genetic basis of ABO antigens has been elucidated; genes determining A and B blood types encode glycosyltransferases in which a few amino acid substitutions result in differing substrate specificities: the A enzyme for UDP-GalNAc and the B enzyme for UDP-Gal, whereas the gene for the O blood type encodes an inactive protein<sup>4</sup>.

Plasma of blood group A individuals contains naturally occurring antibodies to the B antigen. Conversely, that of blood group B individuals contains antibodies to the A antigen. Blood group AB plasma has neither antibodies, and blood group O plasma has both antibodies. Individuals with anti-A and/or anti-B antibodies cannot

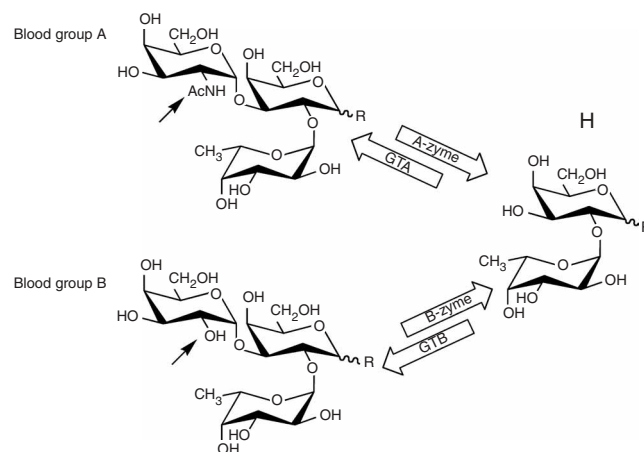
receive a transfusion of blood containing the corresponding incompatible antigen(s). Inappropriate transfusion of ABO-incompatible RBCs can cause complement activation and lysis of the incompatible RBCs, often resulting in an acute intravascular hemolytic transfusion reaction. Inadvertent mismatching of RBCs for ABO blood groups remains one of the most common and persistent causes of serious and sometimes fatal adverse events following transfusion, according to hemovigilance monitoring systems<sup>5,6</sup>. Group O RBCs contain neither A nor B antigens and can be transfused safely into recipients of any ABO blood group, that is, group A, B, AB or O recipients. Thus, type O blood is considered 'universal' and may be used for all RBC transfusions. Hence, blood banks strive to maintain significant inventories of group O RBCs. In reality, however, there is a constant shortage of group O units as these are always in great demand for numerous reasons, including emergency situations in which the patient's blood group is unknown, shortages of blood from a particular blood group, pediatric transfusions, and lack of clarity regarding the patient's blood group (e.g., due to previous transfusions or allogeneic stem cell transplantation). Enzymatic removal of the immunodominant  $\alpha$ GalNAc and  $\alpha$ Gal monosaccharides from group A and B structures of non-O RBCs offers an appealing approach to improve transfusion safety and the overall blood supply<sup>7</sup>.

Goldstein originally envisioned enzymatic conversion of the A and B antigens on RBCs to H antigen by use of exoglycosidases for

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**Figure 1** Structural basis of the ABO blood group antigens. The immunodominant A and B trisaccharide epitopes are formed from the common H disaccharide substrate by  $\alpha$ 1,3-*N*-acetylgalactosaminyltransferase (GTA), defined by the blood group A gene, and  $\alpha$ -galactosyltransferase (GTB), defined by the blood group B gene, respectively. Conversely, the strategy used for enzymatic conversion of blood group A and B antigens to H involves exoglycosidases that specifically hydrolyze the  $\alpha$ 1,3GalNAc ( $\alpha$ -*N*-acetylgalactosidase, A-zyme) or the  $\alpha$ 1,3galactose ( $\alpha$ -galactosidase, B-zyme) to form the common H structure found on O RBCs. Black arrows indicate the different C-2 *N*-acetyl group of GalNAc and OH group of Gal in the immunodominant A and B epitopes, respectively. The immunodominant epitopes are positioned at the termini of oligosaccharide chains on glycolipids and glycoproteins as indicated by R. Increased complexity in ABH oligosaccharide structures are provided by the oligosaccharide carrier chain (R). On human RBCs most structures are based on type 2 polylactosamine chains with repeating Gal $\beta$ 1-4GlcNAc disaccharide units (both glycolipids and N-linked glycoproteins). A minor amount of type 1 chain ABH structures with Gal $\beta$ 1-3GlcNAc are found as glycolipids adsorbed from plasma<sup>3</sup>. A fraction of the blood group A glycolipids with type 2 chain core structures are extended by a  $\beta$ Gal residue (Gal $\beta$ 1-3GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-R), which is further preferentially modified to an A-associated H epitope in subgroup A<sub>2</sub> RBCs (Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-R) and a repetitive A epitope (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-R) in subgroup A<sub>1</sub> RBCs (see **Fig. 4** for glycolipid structures). Cleavage of the repetitive A structure by an  $\alpha$ -*N*-acetylgalactosaminidase will result in exposure of the H disaccharide epitope.



selective removal of  $\alpha$ GalNAc and  $\alpha$ Gal residues of the immunodominant A and B trisaccharide antigens, respectively, to expose the underlying H antigen<sup>8</sup> (**Fig. 1**). For conversion of group B RBCs, he used an  $\alpha$ -galactosidase derived from green coffee beans (*Coffea canephora*), which has a very low pH optimum (pH 3.5) and required as much as 1–2 g enzyme per unit of group B RBCs for adequate conversion at acidic pH 5.5. A phase 2 crossover trial was completed with full unit (~200–220 ml packed) transfusions of group B RBCs enzyme-converted to group O (B-ECO), in which the converted cells functioned and survived normally in group A and O patients<sup>9</sup>. Although this study clearly demonstrated that enzymatic conversion of RBCs is feasible and that B-ECO cells can function as well as

ABO-matched untreated cells in transfusions, a major obstacle to clinical implementation of this method is the large quantities of enzyme required. Other investigators have explored different  $\alpha$ -galactosidases and protocols for conversion of group B RBCs but without substantial improvements in enzyme consumption<sup>10,11</sup>.

Successful conversion of group A RBCs has not yet been achieved. The biochemistry of A antigens is more complex than that of B antigens. Subgroup A<sub>1</sub> RBCs have ~5 $\times$  more A antigens than subgroup A<sub>2</sub>, and, in addition, subgroup A<sub>1</sub> cells have glycolipids with a repeated A structure, which in A<sub>2</sub> cells mainly exist as an A-associated H structure (see **Fig. 1** and **Table 1** for structures)<sup>12,13</sup>. Conversion of only the A<sub>2</sub> subgroup to group O was achieved with a

**Table 1** Substrate specificities analyzed by TLC assay

		$\alpha$ - <i>N</i> -acetylgalactosaminidase	$\alpha$ -galactosidases		
Substrate	Blood group specificity	<i>E. meningosepticum</i> (pH 6.8)	Coffee bean <sup>a</sup> (pH 5.5)	<i>S. griseoplanus</i> <sup>a</sup> (pH 6.8)	<i>B. fragilis</i> (FragA) (pH 6.8)
<b>GalNAc<math>\alpha</math>1-2(3,4)Gal Structures</b>					
GalNAc $\alpha$ 1-2Gal $\beta$ 1-MCO		+	ND	ND	ND
GalNAc $\alpha$ 1-3Gal $\beta$ 1-MCO	A-di	+	ND	ND	ND
GalNAc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-MCO	Linear A-tri	+	ND	ND	ND
GalNAc $\alpha$ 1-4Gal $\beta$ 1-4Glc $\beta$ 1-MCO		+	ND	ND	ND
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal	A-tri	+	ND	ND	–
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GalNAc $\beta$ 1-AMC	A-tetra (type 3 chain) <sup>b</sup>	+ (0.25 U/mg) <sup>c</sup>	–	–	–
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-AMC	A-hepta (type 3 chain, repetitive A)	+ (0.25 U/mg)	ND	ND	ND
<b>Gal<math>\alpha</math>1-3Gal Structures</b>					
Gal $\alpha$ 1-3Gal	B-di	ND	ND	ND	–
Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc	Linear B	ND	+	–	–
Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal	B-tri	–	+	+	+
Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-AMC	B-tetra (type 2 chain)	–	+ (0.017 U/mg)	+	+ (6.6 U/mg)
<b>Gal<math>\alpha</math>1-4Gal Structures</b>					
Gal $\alpha$ 1-4Gal	P1	–	+	–	–
Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc	P <sup>k</sup>	–	+	–	–

<sup>a</sup>From published US patent application no. US 20,050,208,655. <sup>b</sup>Core structures of ABH antigens designated type 1–4, as described previously<sup>3</sup>. <sup>c</sup>Specific activity determined using quantification by fluorography of TLC analysis.

+, complete digestion of substrate using conditions that produce complete digestion of the AMC-labeled blood group A or B tetrasaccharide substrates. –, no detectable digestion of substrate under the same conditions even after extended reaction time (10 $\times$ ). ND, not determined.



chicken liver  $\alpha$ -N-acetylgalactosaminidase using 1 g of enzyme per unit of RBCs at pH 5.5 (ref. 14). An  $\alpha$ -N-acetylgalactosaminidase from *Clostridium perfringens* has also been used<sup>15</sup>. Purification of an  $\alpha$ -N-acetylgalactosaminidase from *Arenibacter latericius* with neutral pH optimum, which appeared to convert both subgroup A<sub>1</sub> and A<sub>2</sub> cells after 24 h incubation, has been reported<sup>16</sup>. The efficiency of the conversion, however, was not confirmed by licensed typing methods, and the gene has not been identified yet. It is, therefore, not clear whether the difficulties with conversion of the A<sub>1</sub> subgroup relate to the complexity of A antigens or to the poor kinetic properties and acidic pH optima of the enzymes used to date<sup>14,15</sup>.

In the past,  $\alpha$ -N-acetylgalactosaminidases and  $\alpha$ -galactosidases used for removing the immunodominant  $\alpha$ GalNAc and  $\alpha$ Gal residues from the A and B antigens, respectively, have mainly been identified and characterized by enzymatic assays using simple monosaccharide *p*-nitrophenyl (pNP) substrates. We hypothesized that the use of this strategy would fail to identify enzymes with selective or exclusive substrate specificities for the more complex branched blood group A and B structures. We therefore screened a large panel of bacterial and fungal isolates to search for enzymes with the preferred substrate specificities and a neutral pH optimum. Here we describe two previously unknown prokaryotic glycosidase families, which contain enzymes with favorable or exclusive substrate specificities for the branched blood group structures, neutral pH optimum, and high efficiency and selectivity in enzymatic conversion of group A, B and AB RBCs to group O RBCs. The  $\alpha$ -N-acetylgalactosaminidase functions with an unusual catalytic mechanism involving NAD<sup>+</sup> and the crystal structure of the enzyme reveals a structural fold that has not been found previously in glycosidases. The availability of these bacterial enzymes should allow efficient and cost-effective enzymatic conversion of blood group A, B and AB RBCs to universal RBCs.

## RESULTS

### Screening for prokaryotic exoglycosidases

To identify enzymes that might have preferred or exclusive specificity for blood group A and B structures and neutral pH optima, we screened a large panel of 2,500 fungal and bacterial isolates for enzymes capable of cleaving A and B oligosaccharide substrates. Most bacteria express high levels of known  $\alpha$ -galactosidases that have acidic pH optima and high activity with the Gal $\alpha$ -pNP substrate, and some also express  $\alpha$ -N-acetylgalactosaminidases that have similarly high activity with the GalNAc $\alpha$ -pNP substrate. We therefore used differential screening with two assays; one with a blood group A or B tetrasaccharide substrate labeled with 7-amino-4-methyl-coumarin (A or B tetrasaccharide-AMC) substrate and one with the GalNAc $\alpha$ -pNP or Gal $\alpha$ -pNP substrate (Table 2). Assays were performed at neutral pH to select enzymes with the preferred pH optimum. Five of the 2,500 isolates were found to display  $\alpha$ -galactosidase activity

with the B tetrasaccharide-AMC substrate and not with the monosaccharide Gal $\alpha$ -pNP substrate, whereas two isolates showed  $\alpha$ -N-acetylgalactosaminidase activity with the A tetrasaccharide-AMC substrate as well as the monosaccharide GalNAc $\alpha$ -pNP substrate (data not shown). Both activities exhibited neutral pH optima.

### Improved enzymes for conversion of group A RBCs – family GH109

The  $\alpha$ -N-acetylgalactosaminidase activities identified in the screen were similar to that of an *Elizabethkingia meningosepticum* (previously termed *Chryseobacterium meningosepticum*, ATCC no. 13253)  $\alpha$ -N-acetylgalactosaminidase (GenBank accession no. AM039444), which was originally identified and cloned using the same blood group A tetrasaccharide-AMC substrate for screening<sup>17</sup>. The deduced protein sequence of the *E. meningosepticum* enzyme was used in GenBank searches to identify a family of enzymes found exclusively in prokaryotes, classified as GH109 in the carbohydrate-active enzyme database (<http://www.CAZY.org/>) (Supplementary Fig. 1 online). The genes encoding five of these proteins were cloned and expressed (Supplementary Methods online). One of two genes identified in *Bacteroides fragilis* (AM039447) as well as those from *Shewanella oneidensis* (AM039445) and *Tannerella forsythensis* (AM039448) were found to encode functional  $\alpha$ -N-acetylgalactosaminidases with properties similar to those of the *E. meningosepticum* enzyme (data not shown). The second gene identified in *B. fragilis* (AM039446) and the *Streptomyces coelicolor* gene (AM039449) did not appear to encode proteins with  $\alpha$ -N-acetylgalactosaminidase activity.

A distant relationship of these enzymes to oxidoreductases was noted using the NCBI conserved domain search. We therefore considered the possibility that these enzymes functioned with NAD<sup>+</sup> and metal ions as cofactors. An N-terminal truncated construct (amino acids 18–444) of the *E. meningosepticum* was expressed efficiently in *Escherichia coli* with a yield of 1 g/l, purified to homogeneity and used for the studies reported here. The purified recombinant enzyme was not affected by EDTA/EGTA chelation of potential metal ions, and exogenous addition of metal ions did not enhance activity. Furthermore, addition of NAD<sup>+</sup> or NADH had no effect on enzyme activity (Supplementary Fig. 2 online). As a result of these findings exogenous addition of metal ions and nucleotides were not used in standard enzyme assays in the study. However, the enzyme does in fact use NAD<sup>+</sup> as a cofactor, but NAD<sup>+</sup> is tightly bound, and the enzyme is saturated in its purified recombinant form.

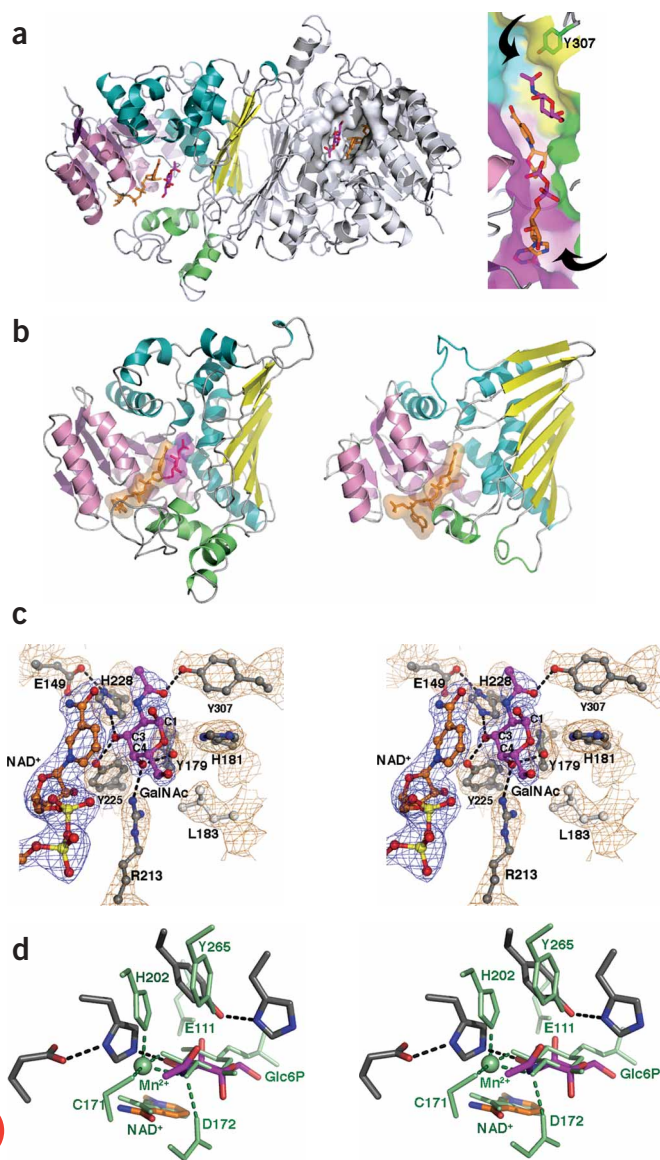
The substrate specificity and kinetic properties of the recombinant *E. meningosepticum*  $\alpha$ -N-acetylgalactosaminidase are presented in Tables 1 and 2. The enzyme has a neutral pH optimum with a relative high specificity for the blood group A AMC substrate (0.25 U/mg) (Supplementary Figs. 3a and 4a online) compared with the monosaccharide GalNAc $\alpha$ -pNP substrate (>10 U/mg).

**Table 2 Kinetic properties of the  $\alpha$ -N-acetyl-galactosaminidase and  $\alpha$ -galactosidase**

Substrate	<i>E. meningosepticum</i> $\alpha$ -N-acetyl-galactosaminidase			<i>B. fragilis</i> $\alpha$ -galactosidase A (FragA)		
	$k_{cat}$ (s <sup>-1</sup> )	$K_m$ (mM)	$k_{cat}/K_m$ (s <sup>-1</sup> mM <sup>-1</sup> )	$k_{cat}$ (s <sup>-1</sup> )	$K_m$ (mM)	$k_{cat}/K_m$ (s <sup>-1</sup> mM <sup>-1</sup> )
Gal $\alpha$ -pNP	0.026 ± 0.001	25.1 ± 1.4	0.0011	0.38 ± 0.01	0.70 ± 0.02	0.54
Gal $\beta$ -pNP	0.010 ± 0.000	3.6 ± 0.3	0.0031	0.00 <sup>a</sup>	NA	NA
GalNAc $\alpha$ -pNP	9.84 ± 0.16	0.077 ± 0.006	127.6	0.00	NA	NA
GalNAc $\beta$ -pNP	0.015 ± 0.000	0.23 ± 0.01	0.087	0.00	NA	NA

Data represent the average of triple or more measurements.

<sup>a</sup>No activity was found with 60×-longer reaction time than was required to readily detect the activity of Gal $\alpha$ -pNP. NA, not applicable.



**Figure 2** Overall structure of the *E. meningosepticum*  $\alpha$ -N-acetylgalactosaminidase. **(a)** Left panel. Cartoon representation of the overall structure of the  $\alpha$ -N-acetylgalactosaminidase dimer. In the left subunit, the dinucleotide binding domain (residues 19–148) is shown in pink, the  $\alpha$ -helices of the C-terminal domain (residues 149–444) in cyan, the  $\beta$ -sheet forming the dimer interface in yellow, and the  $\alpha$ -helical bundle covering the NAD<sup>+</sup> binding tunnel in green. In the right subunit, a partial surface representation shows the entry of the NAD<sup>+</sup> binding tunnel and the active-site pocket. NAD<sup>+</sup> and GalNAc are shown as orange and red sticks, respectively. Right panel. Close-up and clipped view of the NAD<sup>+</sup> binding tunnel created at the interface of the dinucleotide binding domain (pink) and the  $\alpha$ -helical bundle (green). **(b)** Side-by-side representation of  $\alpha$ -N-acetylgalactosaminidase (left) and *Zymomonas mobilis* glucose-fructose oxidoreductase (PDB 10FG) (right), using the same coloring convention as in **a**. The NAD<sup>+</sup> molecule and GalNAc bound to the  $\alpha$ -N-acetylgalactosaminidase and the NADP<sup>+</sup> cofactor bound to 10FG are highlighted. **(c)** Stereo view of the active site of  $\alpha$ -N-acetylgalactosaminidase oriented as in **b**. The GalNAc residue, part of the NAD<sup>+</sup> and the surrounding amino acid residues described in the text are shown as ball-and-stick models. Maximum-likelihood/ $2F_o - F_c$  electron density (contoured at  $\sim 1.5 \sigma$ ) is shown in blue for GalNAc and NAD<sup>+</sup> and in orange for protein residues. **(d)** Stereo view of the superimposition of the active sites of  $\alpha$ -N-acetylgalactosaminidase (gray) and *B. subtilis* 6-phospho- $\alpha$ -glucosidase (green) (PDB 1NRH).

oxidoreductases, lack of similarity to known glycosidases and the unusual enzymatic properties motivated us to determine the structure of the enzyme by X-ray crystallography.

### Structural architecture of the $\alpha$ -N-acetylgalactosaminidase

The crystal structure of the *E. meningosepticum*  $\alpha$ -N-acetylgalactosaminidase was solved by the multiple anomalous dispersion (MAD) method, taking advantage of the anomalous dispersion of selenium, and refined against native data to 2.3 Å resolution (**Supplementary Table 1** online). The structure of a complex with GalNAc was refined to 2.4 Å resolution. The enzyme is present as a homodimer in the crystal and appears to form a dimer in solution, as evidenced by dynamic light-scattering analysis. However, analysis by size-exclusion chromatography suggests that the enzyme exists as a monomer or a dimer, depending on buffer conditions (data not shown). The final structure of the enzyme comprises the entire mature protein (residues 18–444) and a molecule of NAD<sup>+</sup>. Although the distant sequence relatedness with oxidoreductases suggested that the enzyme could function with NAD<sup>+</sup> as a cofactor, similarly to enzymes of the GH4 family<sup>19–21</sup>, this was a surprise because we had observed no stimulation of activity with exogenous addition of NAD<sup>+</sup> or NADH in our studies of the kinetic properties of the enzyme (**Supplementary Fig. 2** online).

Structurally, the protein consists of two independent and closely associated domains, forming a narrow tunnel in which the NAD<sup>+</sup> molecule is embedded (**Fig. 2a**). The N-terminal domain forms a classical dinucleotide-binding Rossmann fold<sup>22</sup>, whereas the C-terminal domain consists of an  $\alpha/\beta$  domain with a large open-faced, seven-stranded, anti-parallel  $\beta$ -sheet, adjacent to nine  $\alpha$ -helices and an  $\alpha$ -helical bundle covering the dinucleotide-binding tunnel. The open-faced  $\beta$ -sheet, with a surface area of 2,800 Å<sup>2</sup>, representing 15% of the total surface of a monomer, makes up the dimer interface. The NAD<sup>+</sup> molecule, copurified with the recombinant enzyme, is anchored within a 10 Å long, narrow tunnel, almost entirely shielded from the solvent; the nicotinamide ring is located in a narrow pocket situated at the bottom of a large depression at the surface of the protein. Because exogenous addition of NAD<sup>+</sup> did not stimulate the enzyme, we developed a method for depletion of NAD<sup>+</sup> by precipitating the protein with ammonium sulfate under acidic

Surprisingly, low but detectable activity was also observed with GalNAc $\beta$ -pNP as well as with Gal $\alpha$  and Gal $\beta$ -pNP substrates (**Supplementary Fig. 5** online). Interestingly, the apparent  $K_m$  value for GalNAc $\alpha$ -pNP is similar to that of GalNAc $\beta$ -pNP, although  $k_{cat}$  values differ more than 1,000-fold. In contrast, the  $K_m$  value for Gal $\beta$ -pNP is over tenfold higher than that for the GalNAc counterpart. Although glycoside hydrolases are usually extremely specific for the anomeric configuration of the glycosidic bond, a member of glycosidase family GH4, namely *Fusobacterium mortiferum* phospho- $\alpha$ -glucosidase, has been reported to be able to cleave both  $\alpha$ - and  $\beta$ -anomers of the nonnatural *p*-NP 6-phosphoglycosides substrates<sup>18</sup>. We did not observe any cleavage of the natural globoside (Gb<sub>4</sub>) glycolipid structure with a terminal  $\beta$ 1-3GalNAc residue; however, low but detectable cleavage of the disaccharide substrate GalNAc $\beta$ 1-4Gal $\beta$ 1-O(CH<sub>2</sub>)<sub>8</sub>COOCH<sub>3</sub> was observed (data not shown). Monitoring the hydrolysis of GalNAc $\alpha$ -pNP by NMR showed that the  $\alpha$ -N-acetylgalactosaminidase functions with a mechanism leading to overall retention of the anomeric configuration (**Supplementary Results** online). The combined findings of sequence relatedness to

conditions. The release of  $\text{NAD}^+$  was confirmed by high-performance liquid chromatography analysis using standards (data not shown). The acid ammonium-sulfate-treated enzyme was inactive after resolubilization; complete activity was restored by exogenous addition of  $\text{NAD}^+$ , but not by related compounds including NADH (Supplementary Fig. 6 online). The same dependence of  $\text{NAD}^+$  was also found for the activity with GalNAc $\beta$ -pNP, confirming that this is an intrinsic activity of the enzyme (Supplementary Fig. 7 online).

The overall fold of the  $\alpha$ -N-acetylgalactosaminidase is unique among the more than 370 glycosidases with known three-dimensional structures (carbohydrate-active enzyme database at <http://www.CAZY.org/>). A Dali search<sup>23</sup> for structural homologs within the nonredundant set of protein structures from the Protein Data Bank revealed that the closest structural relatives belong to the Gfo/Idh/MocA oxidoreductase family (Z-score of 29.4 and r.m.s. deviation of 3.0 Å for 329 equivalent C $\alpha$ -atoms for the closest structural neighbor, namely *Zymomonas mobilis* glucose-fructose oxidoreductase, 1OFG<sup>24</sup>) (Fig. 2b). More distant structural homologs are all identified by means of the classical Rossmann fold. The structural similarity extends to the level of the active-site architecture, where the spatial arrangement of the cofactor and several key residues is conserved, suggesting a common ancestor that has evolved its  $\text{NAD}^+$ -based molecular mechanism to adapt to diverse metabolic requirements.

#### Active site of the $\alpha$ -N-acetylgalactosaminidase

The complex with the reaction product, GalNAc, showed the carbohydrate inside a narrow pocket situated in the middle of a large crater. The wide opening of the crater appears suitable for the accommodation of large aglycon structures such as the blood group A antigen. The GalNAc moiety is found close to  $\text{NAD}^+$  with the C-3 atom of the sugar 3 Å away from the C-4 atom of the nicotinamide ring (Fig. 2c). Four residues, Tyr307, Tyr225, His228 and Glu149, which appear to be important for the interactions with the carbohydrate (Supplementary Fig. 8 online), are invariant within the GH109 family (Supplementary Fig. 9b online).

What governs the preference of the enzyme for GalNAc over Gal? Replacement of the C-2 acetamido group by a hydroxyl substantially impairs enzyme-substrate interaction, as suggested by a 2,000-fold reduction in  $k_{\text{cat}}$  for the hydrolysis of Gal $\alpha$ -pNP compared with GalNAc $\alpha$ -pNP and by a more than tenfold increase in  $K_m$  (Table 2). The structure of  $\alpha$ -N-acetylgalactosaminidase in complex with GalNAc shows that the interaction of Tyr307 with the carbonyl oxygen of the acetamido group of the substrate is probably responsible for the preference for GalNAc substrates.

Our finding that the second family member from *B. fragilis* (AM039446) did not exhibit  $\alpha$ -N-acetylgalactosaminidase activity suggests that the family includes enzymes acting on different substrates. Alternative substrate specificity might originate from chemical variability at C-4, C-5 and/or C-6, as  $\text{NAD}^+$  and the invariant residues impose invariant interactions with the C-2 and C-3 substituents of the carbohydrate. Indeed, in the complex structure, OH-4 establishes hydrogen bonds with the conserved Tyr179 and residue Arg213, whereas no hydrogen bonding partner could be observed for OH-6. This exo-cyclic hydroxyl is surrounded by residues Leu183, Val186, Glu209 and Arg213. Whereas Leu183 is strictly conserved in all members of GH109, the three latter residues are conserved only in the branch of the evolutionary tree comprising *E. meningosepticum* and the enzymes from *Flavobacterium* sp. MED217 and *Robigitalearia biformata* HTCC2501 (Supplementary Figs. 1a and 9a online). These three enzymes are also characterized by a ~15-residue insertion at the tip of the  $\alpha$ -helical bundle.

#### The catalytic mechanism of the $\alpha$ -N-acetylgalactosaminidase

Although the  $\alpha$ -N-acetylgalactosaminidase and the glycosidases from family GH4 differ in their overall protein folds, their active-site architectures show striking similarities (Fig. 2d), thereby constituting a beautiful example of convergent evolution. The glycoside hydrolase family GH4 groups together  $\alpha$ - and  $\beta$ -glycosidases that have been shown recently to use an  $\text{NAD}^+$ -dependent mechanism that is highly unusual for glycosidic bond cleavage<sup>19–21</sup>. The structural superimposition of the active site of  $\alpha$ -N-acetylgalactosaminidase onto those of *Bacillus subtilis* phospho- $\alpha$ -glucosidase, GlvA<sup>20</sup> and *Thermotoga maritima* 6-phospho- $\beta$ -glucosidase, BglT<sup>25</sup>, reveals a perfect overlap of the dinucleotide cofactors, the sugar rings and a tyrosine residue.

Family GH4 enzymes contain a divalent metal ion coordinated by a conserved cysteine, a conserved histidine and the nicotinamide group and thought to stabilize an enediolate-reaction intermediate<sup>20</sup>. Notably, the crystal structure of the  $\alpha$ -N-acetylgalactosaminidase is devoid of any metal ion, in agreement with the observation that the enzyme does not display any metal ion requirements (Supplementary Fig. 2 online). Monitoring of the reaction catalyzed by  $\alpha$ -N-acetylgalactosaminidase indicated that the enzyme proceeds with a mechanism that leads to retention of the anomeric configuration and concomitant exchange of the GalNAc H-2 atom for a solvent proton (Supplementary Results online). These results parallel exactly those of family GH4 glycosidases and therefore indicate that GH109 enzymes most likely operate by a similar mechanism. In this mechanism, an 'onboard'  $\text{NAD}^+$  molecule oxidizes the substrate at C-3, thereby acidifying the proton at C-2. Deprotonation of C-2 by an enzymatic base with concomitant elimination of the glycosidic oxygen generates a 1,2-unsaturated intermediate. The reaction is completed by addition of water to the Michael-like acceptor and reduction of the resulting ketone by the onboard NADH molecule, which returns to the initial  $\text{NAD}^+$  state, ready for another catalytic cycle (Supplementary Fig. 10 online). This mechanism, found first in family GH4 enzymes, allows cleavage of thioglycosides<sup>26</sup>, a feature that is extremely rare among 'classical' glycosidases. Thin layer chromatography (TLC) analysis showed that  $\alpha$ -N-acetylgalactosaminidase is indeed able to cleave benzyl-1-thio- $\alpha$ -N-acetylgalactosaminide (data not shown).

During the reaction catalyzed by GH4 glycosidases, departure of the aglycone is thought to be acid catalyzed<sup>20,25</sup>, but the role and identity of the acid catalyst is still uncertain. Interestingly, the structure of the  $\alpha$ -N-acetylgalactosaminidase does not reveal any suitable residue to protonate the interosidic atom and suggests that this step is perhaps unnecessary in family GH109. This is perfectly compatible with the observed activity with benzyl-1-thio- $\alpha$ -N-acetylgalactosaminide and provides a structural explanation of the hydrolysis of both  $\alpha$ - and  $\beta$ -anomers of pNP substrates; indeed only a minor adjustment of the side chain of His181 is necessary to accommodate a  $\beta$ -linked substrate.

After family GH4, family GH109 constitutes the second glycosidase family that displays structural and mechanistic relatedness to oxidoreductases. There is nothing in the  $\alpha$ -N-acetylgalactosaminidase sequence that suggested hydrolytic activity on glycosides. In consequence it is conceivable that other distant homologs of known oxidoreductases may also correspond to glycosidases and the repertoire of this category of enzymes might further develop. We note a very distant but intriguing similarity between family GH109 members and a recently discovered *T. forsythensis* sialidase<sup>27</sup>.

#### Improved enzymes for conversion of group B RBCs – family GH110

Of the five isolates found to express  $\alpha$ -galactosidase activity with the B tetrasaccharide-AMC at neutral pH, one isolate (no. 2,357) from a



**Table 3** Blood group typing of ECO RBCs with enzyme dose titrations

ECO RBCs	A-zyme dose/ml pRBCs (mg)	B-zyme dose/ml pRBCs (mg)	ABO typing results <sup>a</sup>							
			Ortho anti-A		ES-15		Ortho anti-B		Diagast anti-B	
			IS	4 °C	IS	4 °C	IS	4 °C	IS	4 °C
A <sub>1</sub> -ECO RBCs	0.73		0	0	0	0				
	0.60		0	0	0	0				
	0.50		0	0	0	0				
	0.37		0	0	0	0				
	0.23		0	0	Vw+	0				
	0.13		0	0	W+	W+				
	0.03		0	0	1+	1+				
B-ECO RBCs		0.02					0	0	0	0
		0.01					0	0	0	0
		0.005					0	0	vw	w
		0.0025					0	w	1	2
		0.0013					0	2	1	3
		0.0006					w	2	2	3
A <sub>1</sub> B-ECO RBCs	0.73	0.02	0	0	0	0	0	0	0	0
	0.60	0.01	0	0	0	0	0	0	0	0
	0.50	0.005	0	0	0	0	0	0	0	0
	0.37	0.0025	0	0	0	0	0	vw+	w+	w+
	0.23	0.0013	0	0	0	0	0	w+	w+	1+
	0.13	0.0006	0	0	vw+	w+	0	1+	1+	1+
	0.03	0.0003	1+	2+	2+	2+	vw+	2+	1+	2+

<sup>a</sup>Typing with licensed ABO typing reagents and methods as indicated using agglutination score 0 to 4+.  
IS, immediate spin reactions; pRBCs, packed RBCs.

*Streptomyces griseoplanus* strain exhibited the highest enzyme activity in lysates and was found to secrete the  $\alpha$ -galactosidase enzyme into the medium. Initial attempts to purify the enzyme from the medium failed because of an insufficient amount of enzyme, and we therefore screened different nutrient carbon sources using minimal medium in an attempt to enhance secretion. A 10- to 40-fold induction of secretion was achieved with galactose and lactose (Supplementary Methods online), and an enzyme with a molecular weight of ~75 kDa was isolated from the medium. A number of peptide sequences (S1-S7) were obtained by Edman degradation and tandem mass spectrometry, and database searches identified weak sequence similarities with a putative protein from *Streptomyces avermitilis*. Further searches with the *S. avermitilis* sequence identified four additional genes with substantial similarity from *Bacteriodes fragilis* and *Bacteriodes thetaiotaomicron* (Supplementary Figs. 1b and 9b and Supplementary Methods online). All five identified genes were cloned by PCR from their corresponding genomic DNA, expressed in *E. coli* as His<sub>6</sub>-tagged proteins, and shown to encode  $\alpha$ -galactosidases when analyzed with a B tetrasaccharide-AMC substrate (data not shown). The lack of sequence similarity between the newly identified  $\alpha$ -galactosidases and any known glycosidase in the CAZY database allows us to define the glycosidase family GH110. One of the two  $\alpha$ -galactosidase homologs found in *B. fragilis* NCTC 9343 (designated FragA, EMBL Nucleotide Sequence Database accession no. AM109955) was expressed in *E. coli* without its N-terminal signal sequence (amino acids 23–605) with a yield of 500 mg/l, purified to homogeneity, and used for further studies.

The substrate specificity and kinetic properties of the recombinant FragA  $\alpha$ -galactosidase are presented in Tables 1 and 2. The enzyme has a broad pH optimum between 5 and 7.5 with the B tetrasaccharide-AMC substrate (Supplementary Fig. 3b online). Interestingly, the

recombinant FragA enzyme exhibited low activity with the Gal $\alpha$ -pNP substrate (0.3 U/mg) compared to the blood group B substrate (6.6 U/mg) (Table 2), whereas the purified endogenous enzyme from *S. griseoplanus* did not exhibit detectable activity with the Gal $\alpha$ -pNP substrate (data not shown). The substrate specificity was remarkably stringent for  $\alpha$ -1,3-linked galactose in the branched blood group B structure (Table 1 and Supplementary Fig. 4b online). The enzyme cleaved neither  $\alpha$ 4Gal linkages found in P1 and P<sup>k</sup> blood group antigens nor the  $\alpha$ 3Gal linkage in the linear B structure without fucose (the so-called Galili antigen). The FragA  $\alpha$ -galactosidase has more than 300-fold higher activity with the blood group B tetrasaccharide-AMC substrate than the coffee bean enzyme, whereas the inverse (100-fold) is the case for the Gal $\alpha$ -pNP substrate (Tables 1 and 2). One  $\alpha$ -galactosidase with neutral pH optimum has been isolated previously from the marine bacterium *Pseudoalteromonas* sp. (KMM 701) with a specific activity of 9.8 U/mg with the Gal $\alpha$ -pNP substrate<sup>11</sup>, but given the different properties reported, this enzyme is probably unrelated to the genes described here. We are attempting to crystallize the  $\alpha$ -galactosidase to further elucidate the catalytic mechanism of this enzyme family.

### Enzymatic conversion of blood group A, B and AB RBCs at neutral pH

The two glycosidase families described here contain enzymes that have highly suitable kinetic properties for enzymatic removal of A and B antigens from the surface of RBCs. We first developed a buffer system for enzymatic conversion of RBCs with the recombinant *E. meningosepticum*. The recombinant enzyme has an isoelectric point of 7.6 as shown by isoelectrofocusing, and analysis of the electrostatic surface of the crystal structure indicated that the protein surface near the catalytic region is slightly positive. We therefore hypothesized that



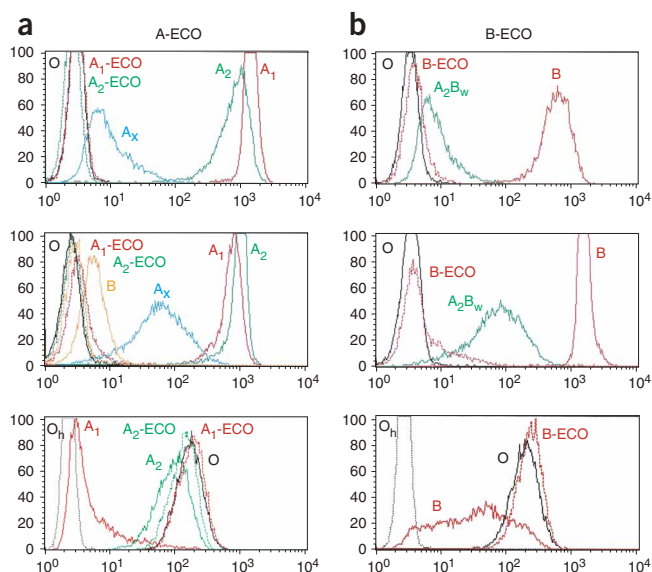
the enzyme at neutral pH could interact with the strong negative charges of primarily sialic acids on the surface of RBCs leading to local concentration of the enzyme at substrate sites. Enzyme conversion was performed at low ionic strength in glycine buffer with varying concentrations of NaCl. Complete removal of A antigens was achieved in 200 mM glycine (pH 6.8) with 3 mM NaCl as evaluated by agglutination typing with routine licensed reagents and methods as well as by sensitive fluorescence-activated cell sorting (FACS) analysis (Table 3 and Fig. 3). In this buffer system >90% of the enzyme was associated with the RBCs after centrifugation. In contrast, increasing the NaCl concentration to 10 mM or more resulted in release of the enzyme from the RBCs. In agreement with our hypothesis, increased NaCl also negatively affected the conversion efficiency, showing that the interaction is important for efficiency (data not shown). Importantly, this feature also allowed a simple and efficient wash-removal process of the enzyme involving three wash cycles in isotonic PBS. The residual enzyme load associated with washed cells was consistently less than 40 ng/ml of packed RBCs as measured with a sensitive capture enzyme-linked immunosorbent assay using total detergent lysates of cells (data not shown). The conversion process is linear with enzyme dose and time, and has a low enzyme consumption of 300 µg/ml packed RBCs for 60 min of incubation. RBCs from more than 200 group A<sub>1</sub> and A<sub>2</sub> donor units have been analyzed and all were converted efficiently. Approximately 60 mg enzyme is required to convert one unit (~200 ml packed RBCs) of blood group A<sub>1</sub> cells. Conversion of the weak blood group A<sub>2</sub> subgroup, however, requires only 15 mg enzyme. The conversion process does not affect physiologic and metabolic parameters of RBCs, including osmotic fragility, levels of 2,3-DPG, ATP and methemoglobin (data not shown).

We selected the recombinant Fraga  $\alpha$ -galactosidase because it has a slightly basic isoelectric point similar to the *E. meningosepticum* enzyme; using the same glycine buffer system as for group A RBCs, we effectively produced, by enzymatic conversion of blood group B RBCs with the Fraga enzyme, B-ECO RBCs that type as group O by licensed reagents and methods used in clinical blood bank practice (Table 3). Similar to our findings with the A conversion process, the low ionic strength is required for efficient conversion, and increasing NaCl concentration in the glycine buffer or use of other buffer systems with higher ionic strength such as PBS and phosphate-citrate decreases the efficiency. The conversion process is linear with enzyme dose and time, and has a very low enzyme consumption of 10 µg/ml packed RBCs for 60 min incubation period. RBCs from more than 50 group B donors have been analyzed in repeat studies and all were converted efficiently with 10 µg/ml packed RBCs (data not shown). Thus, less than 2 mg enzyme are required to convert one unit of blood group B cells. Because the two enzymes used for conversion of A and B RBCs were selected for similar properties and function efficiently in the same conversion buffer system, we were able to convert blood group AB RBCs efficiently both by sequential enzyme digest as well as with a one-pot enzyme digest with the  $\alpha$ -N-acetylgalactosaminidase and  $\alpha$ -galactosidase (Table 3).

The efficiency of the conversion processes was evaluated by sensitive FACS analysis using anti-A and anti-B reagents (Fig. 3). FDA-licensed anti-A blood grouping reagents showed complete removal of A antigens on a homogenous population of RBCs. The high sensitivity of the assay was demonstrated by clear detection of the low amount of A antigen present on the weak A<sub>x</sub> subgroup RBCs. We also included a highly sensitive anti-A monoclonal antibody, ES-15, that detects the minimal amount of A antigen, which is present naturally on group B RBCs<sup>28</sup>. ES-15 clearly detected both the A antigen on A<sub>x</sub> subgroup RBCs and on group B RBCs. The ES-15 reactivity with B RBCs was

sensitive to treatment with the  $\alpha$ -N-acetylgalactosaminidase, confirming that the antibody does detect the minor amounts of A antigens on B RBCs. The ES-15 reagent, but not licensed anti-A reagents, did show a tendency toward a shift in the histogram curve (Fig. 3a, middle panel) of A-ECO RBCs, indicating that miniscule amounts of A antigens at a level considerably lower than on group B RBC could be detected on A<sub>1</sub>-ECO but not on A<sub>2</sub>-ECO RBCs. Similarly, FDA-licensed anti-B reagents showed a homogeneous B-ECO cell population almost nonreactive with anti-B. The high sensitivity of this assay was demonstrated by including AB<sub>weak</sub> subgroup cells, on which the weakly expressed B antigens were readily detected. Several licensed anti-B typing reagents were tested (data not shown), and one CE-approved anti-B blood grouping reagent, Diagast, was found to exhibit higher sensitivity with the AB<sub>weak</sub> cells and a minor proportion of B-ECO cells were weakly labeled by the Diagast anti-B antibody, although at a level lower than the weak AB<sub>weak</sub> subgroup cells. Analysis with an anti-H antibody was included to demonstrate appearance of the appropriate H antigen product after removal of either  $\alpha$ GalNAc or  $\alpha$ Gal terminal residues, and both A-ECO and B-ECO RBCs expressed H similar to group O RBCs as expected (Fig. 3, lower panels).

Further biochemical analysis of ECO cells included TLC of glycolipids as well as SDS-PAGE western blot of total membrane (glyco)-protein extracts. TLC of glycolipid extracts revealed essentially complete conversion of blood group A and B glycolipid species to the corresponding H species, including the major monosialyl A and B gangliosides designated G9A and G9B (Fig. 4). This was further confirmed by mass spectrometric analysis of the glycolipid fractions migrating in the region of the most abundant A-active glycolipid species, designated A<sup>a</sup> and A<sup>b</sup>, which showed a complete absence of the A glycolipid species in extracts from converted RBCs and the presence of only the corresponding H<sub>1</sub> and H<sub>2</sub> products (data not shown). The blood group A antigens are believed to be more complex than B antigens in that a fraction of the A active glycolipids have a repeated A structure<sup>13</sup>. This structure has been found only on glycolipids and not on glycoproteins. Cleavage of the terminal GalNAc residue of these glycolipids will result in exposure of an H structure extended from an internal A structure in H-A<sup>a</sup> and H-A<sup>b</sup> glycolipids (Fig. 4). Both subgroups A<sub>1</sub> and A<sub>2</sub> RBCs contain these glycolipids, and after enzyme conversion the A<sub>1</sub> subgroup RBCs will contain more than the A<sub>2</sub> subgroup. This H antigen resembles the mucin-type H structure (Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\alpha$ 1-O-Ser/Thr) expressed in epithelial tissues of all ABO groups<sup>29</sup> and is not reactive with typing anti-A antibodies. It is therefore our hypothesis that these A-associated H-A structures found only on glycolipids do not represent antigens recognized by human anti-A immunity, but clinical studies are required to address this. The analysis of glycolipids from converted cells also showed that the *E. meningosepticum*  $\alpha$ -N-acetylgalactosaminidase does not cleave the major glycolipid globoside (Gb<sub>4</sub>, blood group P antigen terminating by a GalNAc $\beta$ 1-3 linkage) in RBC membranes (Fig. 4), despite the finding that the enzyme has low but detectable activity with GalNAc $\beta$ -pNP (Table 2). The glycolipid analysis clearly demonstrated the effectiveness of the process as well as how selective the process is in digesting only the blood group A and B terminating glycolipids. Western blot with the sensitive ES-15 anti-A and the Diagast anti-B monoclonal antibodies revealed very faint diffuse staining of membrane glycoproteins of A-ECO and B-ECO RBCs, respectively (data not shown), which is in agreement with the findings that FACS analysis with the most sensitive monoclonal antibody reagents reveal minute amounts of residual A and B antigens (Fig. 3).



**Figure 3** FACS of native and ECO RBCs. Histograms show the degree of staining of RBCs with different blood grouping reagents. The x-axis represents the fluorescence intensity on a logarithmic scale whereas the y-axis shows the number of RBCs evaluated. Solid lines represent untreated native RBCs and dashed lines in the same color are the enzyme-treated RBCs from the same individual (see color code below). The dotted black line in the H-antigen staining histogram (lower panels) represents the negative control, RBCs from a Bombay ( $O_h$ ) donor lacking any A, B or H antigen on RBCs. To facilitate interpretation of the histograms, enzyme-treated group O and subgroup RBC were not included. However, their curves completely overlap the result for native group O (data not shown). **(a)** RBCs were stained with licensed monoclonal anti-A blood grouping reagent (upper panel), the monoclonal ES-15 anti-A research reagent (middle panel) and direct-conjugated monoclonal anti-H (lower panel). Red, blood group A<sub>1</sub>; green, A<sub>2</sub>; blue, A<sub>x</sub>; orange, B; black, O. The weak A<sub>x</sub> subgroup control was from a serologically and genetically characterized test RBC donor with the A<sup>K</sup>-1 O<sup>J</sup> genotype. Group B RBCs were tested only with ES-15 as licensed anti-A reagents do not detect any A antigen on their surface. **(b)** RBCs were stained with the monoclonal licensed anti-B blood grouping reagents from Ortho (US FDA-licensed) (upper panel), Diagast (CE-approved in European Union) (middle panel), and direct-conjugated monoclonal anti-H (lower panel). Red, blood group B; green, subgroup A<sub>2</sub>B<sub>w</sub>; black, O.

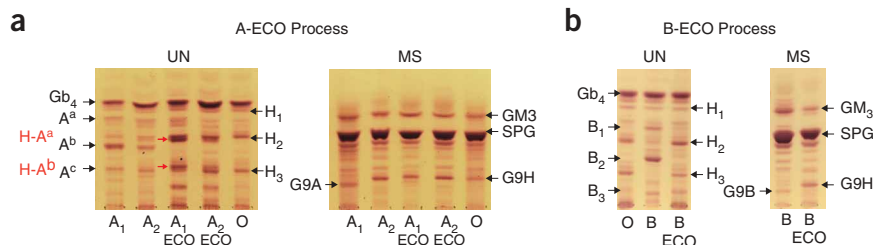
## DISCUSSION

The present study identified two prokaryotic glycosidase gene families, not previously described, containing  $\alpha$ -N-acetylgalactosaminidase (GH109) and  $\alpha$ -galactosidase (GH110) activities with neutral pH optima and high activity with blood group A and B substrates, respectively. The GH109  $\alpha$ -N-acetylgalactosaminidase family represents to our knowledge the only known source of enzymes suitable for efficient enzymatic conversion of blood group A RBCs. The GH110  $\alpha$ -galactosidase family has unprecedented restricted specificity for blood group B substrates. The enzymes are expressed with high yields in *E. coli* and because they have similar properties, a single common conversion buffer system and process can be used to remove A and B antigens and produce ECO RBCs from A, B and AB RBCs that type as blood group O with routine licensed typing reagents and methods. Extensive FACS and biochemical analyses confirm the efficient removal of the immunodominant A and B antigens and exposure of the underlying H antigens. The current process, which is performed manually at neutral pH, is scalable to automated full-unit conversions, and ECO cells produced by this method are predicted to

survive and function in a manner equivalent to native group O RBCs in non-ABO matched individuals as reported previously for B-ECO RBCs<sup>9</sup>. The process has a projected consumption of ~60 mg (A-ECO) and 2 mg (B-ECO) recombinant enzyme with 60-min enzyme treatment per unit RBCs. This is ~30- (A-ECO) and 1,000-fold less (B-ECO) enzyme than the conversion protocol developed for group B RBCs with the Coffee bean  $\alpha$ -galactosidase<sup>9</sup>. Accordingly, we believe that automated cost-effective processes can be developed for practical use in transfusion medicine.

Glycosidases are grouped in over 100 sequence-based families that have been shown to correlate with the 3-D structure and molecular mechanisms of catalysis<sup>30,31</sup>. The glycosidase families identified in this study exhibit no substantial sequence similarity to any other glycosidase families, including families GH4, GH27, GH36 and GH57 (CAZY database at <http://afmb.cnrs-mrs.fr/CAZY/>), which contain all previously reported  $\alpha$ -N-acetylgalactosaminidases and  $\alpha$ -galactosidases, including the two most studied enzymes for blood group B red-cell conversion derived from green coffee and from soy beans<sup>32,33</sup>, as well as enzymes used previously to convert weak blood group A<sub>2</sub> RBCs<sup>1,4,34</sup>.

**Figure 4** TLC analysis of native and ECO RBC glycolipids. **(a,b)** Total upper neutral (UN) and monosialyl (MS) glycolipid fractions from RBC's were separated on high-performance thin layer chromatography plates. Arrows indicate mobility of major blood group ABH glycolipids as well as Gb<sub>4</sub> (globoside) and SPG (sialyl-paragloboside, NeuAc $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\alpha$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-Cer). The nomenclature of ABH active glycolipids used for type 2 chain ABH active glycolipids is H<sub>1</sub>/B<sub>1</sub>/A<sup>a</sup>, H<sub>2</sub>/B<sub>2</sub>/A<sup>b</sup> and H<sub>3</sub>/B<sub>3</sub>/A<sup>c</sup> with increasing number of Gal $\beta$ 1-4GlcNAc disaccharide units<sup>3</sup>. Although, glycolipid bands separated by TLC may contain multiple species, the H<sub>1</sub> (Fuc $\alpha$ 1-2Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-Ceramide), H<sub>2</sub> (as H<sub>1</sub> with one lactosamine unit Gal $\beta$ 1-4GlcNAc $\beta$ 1-3 inserted) and H<sub>3</sub> (as H<sub>1</sub> with two lactosamine units Gal $\beta$ 1-4GlcNAc $\beta$ 1-3 inserted) bands in O UN clearly represent major distinct bands with different mobilities. The corresponding A (a) and B (b) structures A<sup>a</sup>/B<sub>1</sub>, A<sup>b</sup>/B<sub>2</sub> and A<sup>c</sup>/B<sub>3</sub> GalNAc/Gal $\alpha$ 1-3 added to the penultimate  $\beta$ Gal residues in H structures in A and B UN are similarly distinct bands. Comparison with the profile of ECO reveals disappearance of A and B variants and appearance of the corresponding H bands. This includes the branched G9A/B glycolipid (GalNAc/Gal $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-4GlcNAc $\beta$ 1-3[NeuAc $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6]Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ 1-Ceramide), which is a branched structure with one branch terminated by sialic acid and one branch with an ABH determinant. Group A active glycolipids are more complex than B in that they include the internal A structures A-associated H (designated H-A<sup>a</sup> and H-A<sup>b</sup>), which migrate just below the H<sub>2</sub> and H<sub>3</sub> glycolipids, respectively. The A<sup>b</sup> glycolipid band contains a mixture of type 2 chain A<sup>b</sup> and the repetitive A-A<sup>a</sup> glycolipid (GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-3GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-4GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc $\beta$ 1-Ceramide), whereas the A<sup>c</sup> band contains type 2 chain A<sup>c</sup> and the repetitive A-A<sup>a</sup> glycolipid<sup>3</sup>. The loading corresponds to glycolipid fractions derived from 4 ml of packed RBCs.



The human lysosomal  $\alpha$ -galactosidase and  $\alpha$ -N-acetylgalactosaminidase are close homologs to each other and are both members of family GH27. All of these enzymes are characterized by having exclusively a classical catalytic machinery and molecular mechanism leading to retention of the anomeric configuration<sup>35,36</sup>, with the exception of family GH4 enzymes that use NAD<sup>+</sup> and Mn<sup>2+</sup> as cofactors<sup>19–21</sup>. The  $\alpha$ -N-acetylgalactosaminidase family described here is unusual in that it uses an NAD<sup>+</sup>-based mechanism, similarly to family GH4 enzymes. Interestingly, the proposed mechanism of the  $\alpha$ -N-acetylgalactosaminidase is not prone to transglycosylation, a known side reaction of retaining glycosidases, which can fortuitously give rise to nonnatural structures at high substrate concentration.

Preferred properties of an exoglycosidase suitable for enzymatic conversion of RBCs include the following parameters: (i) high substrate specificity for the blood group antigens to restrict the reaction to the immunodominant blood group A and B antigens; (ii) reaction conditions suitable for maintenance of RBC integrity and functions; (iii) high efficiency in cleavage of antigens on the RBC surface to minimize residual antigens and enzyme consumption; and (iv) properties to facilitate enzyme removal from the RBCs by routine cell-washing techniques. The glycosidases presented in this study offer all of these characteristics. In particular, the  $\alpha$ -galactosidases represent, to our knowledge, the only  $\alpha$ 3-linkage-specific exo-galactosidases reported and their specificity for the branched blood group B trisaccharide structure suggests restricted substrate specificity. This has practical implications as human RBCs express other  $\alpha$ Gal-terminating structures, including  $\alpha$ 4Gal residues in the P1 and P<sup>k</sup> blood group antigens, which are cleaved by the  $\alpha$ -galactosidases used in previous studies<sup>32,33</sup>. The two enzyme families described here perform efficiently in conversion of RBCs; ECO cells type as group O with all licensed reagents; and sensitive FACS and glycolipid analyses confirm efficient removal of A and B antigens (Figs. 3 and 4). Finally, enzymes from both families are slightly basic and associate with the negatively charged RBCs through ionic interactions, thereby enabling efficient removal with isotonic buffer solutions, such as PBS, used for cell washing.

The availability of enzymes from these glycosidase families has resulted in the development of a simple and efficient process for producing universal RBCs that type as blood group O. Clinical translation of this approach may allow improvement of the blood supply and enhancement of patient safety in transfusion medicine.

## METHODS

**Enzyme assays.** Structures of substrates are shown in Tables 1 and 2. The AMC derivatives were custom synthesized by Alberta Chemical Research Council. The 8-methoxycarbonyloctyl ((CH<sub>2</sub>)<sub>8</sub>COOCH<sub>3</sub>, MCO) oligosaccharides were obtained from the same source, and other substrates were obtained from Sigma-Aldrich, V-LABS and Oxford Glyco Sciences. Assays with oligosaccharide structures were performed at 26 °C in reaction mixtures of 10  $\mu$ l. Product formation was analyzed by TLC using silica gel-coated TLC plates (Merck). The AMC-labeled tetrasaccharides were used for screening and one unit of enzyme activity was defined as the amount of enzyme required to cleave 1  $\mu$ mole of A or B tetrasaccharide-AMC substrate per minute using a 10- $\mu$ l reaction volume with 100 mM NaPO<sub>4</sub>, pH 6.8, 50 mM NaCl, 0.25 mg/ml of BSA and 0.1 mM substrate. Importantly, calculation of the specific activities with these substrates was based on complete cleavage of the substrate in the reaction volume. Assays with chromogenic pNP substrates were carried out at 26 °C in reaction mixtures of 400  $\mu$ l with 100 mM NaPO<sub>4</sub>, pH 6.8, 50 mM NaCl and 2.5 mM substrate. Reactions were terminated by addition of 600  $\mu$ l 1 M Na<sub>2</sub>CO<sub>3</sub> and pNP formation was quantified at 405 nm ( $\epsilon$  = 18,300 cm<sup>-1</sup> M<sup>-1</sup> at pH > 10). In the chromogenic assays all results were based on reactions with consumption of <10% of the substrate. Determination of kinetic parameters was performed using substrate concentrations of 0.5 to 3–10

times the obtained  $K_m$ . Reactions with the  $\alpha$ -N-acetylgalactosaminidase and Gal $\alpha$ -pNP, however, were carried out using 1–12 mM substrate, which is substantially lower than the apparent  $K_m$ .

**Crystallization and data collection.** Before crystallization the purification buffer was extensively exchanged with 10 mM HEPES buffer at pH 7.5. Recombinant  $\alpha$ -N-acetylgalactosaminidase was crystallized using the hanging drop vapor diffusion method by mixing equal amounts of protein at 21 mg/ml with a solution consisting of 46–50% 2-methyl-2,4-pentanediol (MPD) and 75 mM sodium citrate at pH 6.25. Rod-shaped crystals appeared within 1 d and belong to space group P6<sub>3</sub>22 with cell dimensions of 88  $\times$  88  $\times$  300 Å and one molecule per asymmetric unit. A MAD data set for a selenomethionylated protein crystal, as well as a native data set was collected at beam line ID23-EH1 (ESRF, Grenoble) and a data set for a complex with GalNAc was collected at beam line ID14-EH2 (ESRF, Grenoble). Before data collection, crystals were flash-frozen in a nitrogen gas stream at 100 °K. Data were indexed and integrated with MOSFLM<sup>37</sup> and scaled with the program SCALA<sup>38</sup>. Data collection statistics are summarized in Supplementary Table 1 online.

**Structure solution and refinement.** Data were prepared for phasing with XPREP (Bruker). The selenium substructure was solved with SHELXD<sup>39</sup>, taking advantage of data up to the highest resolution shell, and phasing combined with solvent flattening was carried out with SHELXE<sup>40</sup>, leading to a pseudo-free correlation coefficient of 70.5%. Starting from experimental phases, an initial model comprising 361 residues (out of 429), 257 of which docked into sequence, was automatically built with the ARP/wARP package<sup>41</sup> and completed manually using TURBO-FRODO<sup>42</sup>. The resulting model was refined against native data extending to 2.3-Å resolution with REFMAC<sup>43</sup>. A random set of 4% (1,231) reflections was set aside for cross validation purposes. Automated solvent building was performed with ARP/wARP<sup>41</sup>. For the structure solution of the enzyme-GalNAc complex, the final native model was subjected to rigid body refinement with REFMAC<sup>43</sup> and further refinement was carried out as described above, with 4.1% (1,111) of reflections, taken over from the native data set, set apart for cross validation. The final protein structures encompass residues Lys19 to Tyr444 and have correct stereochemical properties, with all residues contained in the allowed regions of the Ramachandran plot, as verified with WHATCHECK<sup>44</sup>. Refinement and structure quality statistics are listed in Supplementary Table 1 online. Figure 1 was drawn with ChemDraw, CambridgeSoft Corporation, and Figure 2 was generated with PYMOL (DeLano, W.L. The PyMOL Molecular Graphics System (2002) on <http://www.pymol.org/>).

**Enzymatic conversion of RBCs with glycosidases at neutral pH.** Standard enzymatic conversion reactions were performed in 1 ml reaction mixtures containing 200 mM glycine, pH 6.8 and 3 mM NaCl with 30% packed red blood cells and enzyme as indicated. Fresh whole blood was obtained from Oklahoma Blood Institute and buffy coat removed. RBCs were prewashed 1:1 and 1:4 vol/vol in conversion buffer before addition of enzyme. The conversion reaction was incubated for 60 min with gentle mixing at 26 °C, followed by four repeat washing cycles with 1:4 vol/vol of saline by centrifugation at 1,000 r.p.m. The washed enzyme-treated ECO RBCs were ABO-typed according to standard blood banking techniques using licensed monoclonal antibody reagents<sup>45</sup>. Murine monoclonal Anti-A reagent obtained from Ortho. Undiluted, concentrated murine monoclonal anti-A typing reagent supplied for further manufacturing purposes by Celliance. Murine monoclonal Anti-B reagents obtained from Ortho and Diagnost.

**Flow cytometry.** Flow cytometry analysis of native and ECO RBCs was performed using a FACScan flow cytometer (Becton Dickinson) with monoclonal antibody reagents and phycoerythrin-labeled rat-anti-mouse kappa immunoglobulin (Becton Dickinson). An FITC-conjugated monoclonal anti-H (BRIC 231 from BITS/IBGRL) was also used. Briefly, 500,000 RBCs were suspended in PBS in 96-well plates in a 50- $\mu$ l reaction volume. Cells were fixed for 10 min in 0.1% glutaraldehyde in PBS to prevent agglutination of antigen-positive cells. After washing 3 $\times$  in 150  $\mu$ l PBS, cells were again suspended in 50  $\mu$ l PBS, after which 5  $\mu$ l of undiluted primary antibody was added and incubated for 10 min. After three washes and resuspension in 50  $\mu$ l PBS, 5  $\mu$ l of undiluted secondary antibody was added. Cells were then analyzed



after another three washes (as above) and resuspension in 300  $\mu$ l PBS. All incubations were carried out in darkness at 20 °C under gentle agitation. A total of 10,000 events were evaluated and an RBC gate corresponding to ~90% of all cells was set during analysis of the data.

**Glycolipid analysis.** Glycosphingolipids were prepared essentially as previously described<sup>46</sup>. Briefly, RBC membranes were extracted by homogenization in ten volumes of isopropanol/hexane/water (55:25:20, vol/vol/vol), filtered, evaporated and partitioned by Folch extraction method. The upper phase was collected, evaporated, dialyzed and applied to a DEAE Sephadex column. The pass-through fraction contained the total upper neutral lipids, and the monosialyl fraction was eluted with 0.05 M ammonium acetate, evaporated and dialyzed against water to remove acetate. Total upper neutral (UN) and monosialyl (MS) glycolipid fractions were analyzed by high-performance thin-layer chromatography. TLC plates (Merck) were developed using the solvent system chloroform/methanol/0.5 M  $\text{CaCl}_2$  in water (50:40:10, vol/vol/vol). Glycolipids were stained by heating with 0.05% orcinol in 0.5 M  $\text{H}_2\text{SO}_4$ .

**Accession codes.** EMBL Nucleotide Sequence Database: the nucleotide sequences of the cloned genes reported in this paper were deposited with accession nos. AM039444, AM039445, AM039446, AM039447, AM039448 and AM039449 for the  $\alpha$ -N-acetylgalactosaminidases and homologs; AM109953, AM109954, M109955, AM109956 and AM109957 for the  $\alpha$ -galactosidases. Protein Data Bank: coordinates of the crystal structures have been deposited with accession reference nos. 2IXA and 2IXB.

Note: Supplementary information is available on the Nature Biotechnology website.

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#### AUTHOR CONTRIBUTIONS

Q.P.L. contributed to screening and purification of enzymes, planning and design of the project, and manuscript writing; G.S. contributed to the X-ray crystallography and manuscript writing; H.Y. contributed to purification of enzymes; E.P.B. and K.S. contributed to cloning of genes; G.P. contributed to cloning of genes; J.S. and K.S. contributed to development of enzyme conversion protocol; E.N. contributed to glycolipid analysis; S.B.L. contributed with NMR analysis; T.W. contributed to planning and design of the project; J.M.N. and W.S.L. contributed to sequencing of purified enzyme; Y.B. contributed to manuscript writing; M.L.O. contributed to FACS analysis, planning and design of the project and manuscript writing; B.H. contributed to planning and manuscript writing; H.C. contributed to planning and design of the project, and manuscript writing.

#### COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturebiotechnology>.

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## ambr<sup>®</sup> 250 modular

### Performance Data

ambr<sup>®</sup> 250 modular provides a scale down model for bench top and pilot plant scale bioreactors.

ambr 250 modular is a high performance system that is easy to set up and allows users to run multiple parallel bioreactors.

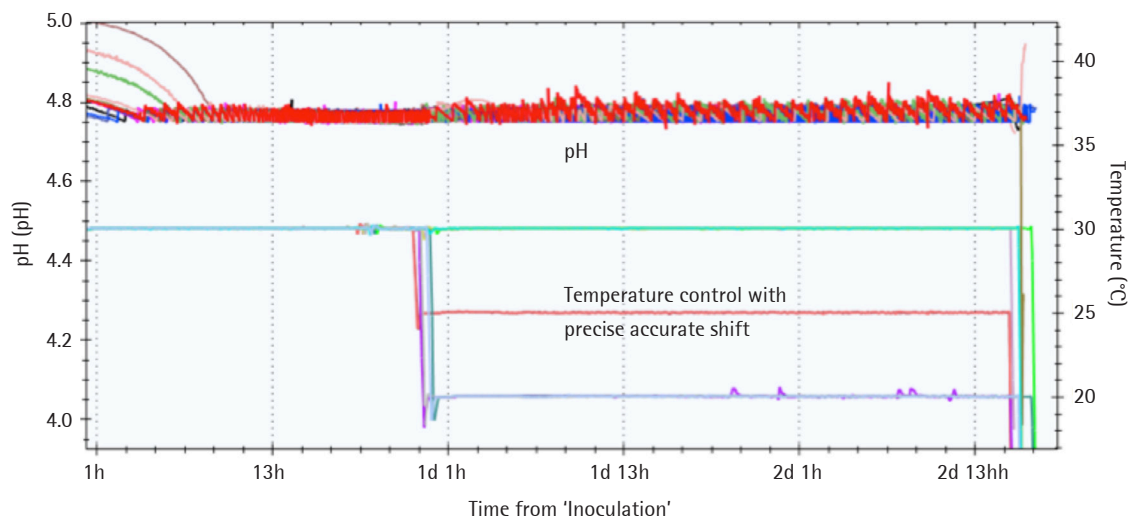


### Precise and Accurate Control

Good control is essential if the ambr 250 modular is to be used for multi parallel experiments, where any process deviation needs to be eliminated.

Figure 1:

Results showing very well controlled process pH and temperature for a high cell density microbial fermentation. Precision and consistency is crucial if experiments are to be run in parallel for comparison.



## Consistent

Due to the precise control of all process parameter, the cultures run under the same condition have very good consistency and repeatability; an essential characteristic for any multi parallel bioreactor system.

Figure 2:  
Results showing DO control of 8 bioreactor system

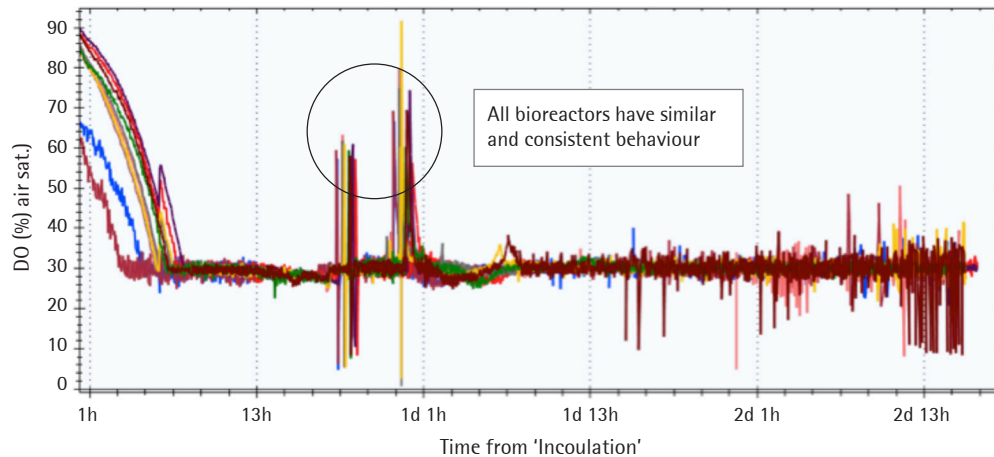
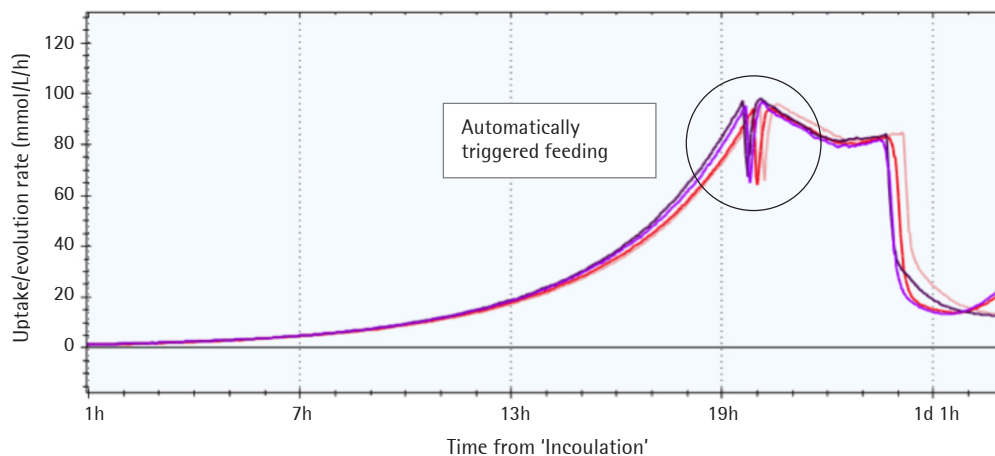


Figure 3:  
Showing carbon dioxide evaluation in 4 bioreactor in parallel



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amb® 250 takes amb technology to the next level with a new platform that adds increased working fermentation volumes as well as individual control on all process parameters. This enhanced environment supports the culture requirements for high demanding microbial fermentation and mammalian cultures and applications where larger sample volumes are required. amb® 250 provides an efficient and rapid high throughput scale-down system at 100 - 250 mL volumes to explore a wide range of conditions and strains with scalability to bench top stirred tank bioreactors.

**amb® 250 provides an efficient, high throughput scale-down model for process development and offers a step change improvement in lab productivity**

- **A better tool for process development**  
amb® 250 dramatically improves productivity and enables full DoE experiments to be performed at a fraction of the cost and with higher throughput than can be done in traditional bench top reactors.
- **Excellent scalability to large scale bench top bioreactors**  
amb® 250 is designed and tested to show that results from this scale-down system are predictive of full size bioreactors and fermenters up to 30 L.
- **Rapid evaluation of a wide range of conditions and cell line/strain performance**  
amb® 250 provides a highly parallel system to rapidly develop processes for clones or strains/lines in a real bioreactor environment with full control of the culture conditions.
- **Reduced infrastructure**  
The low volume, compact, single use, fully disposable reactors remove the need for extensive cleaning and sterilization facilities and minimize the lab footprint. In addition the rapid turnaround means more experiments with less resources.



# ambr® 250 System Combines 12 or 24 'Easy Connect' Single-Use Bioreactors, Fully Automated Platform, Bioreactor Controller and Flexible System Control Software

## **ambr® 250 platform**

Fully automated - including media fill, inoculation, sampling and feeding with minimal operator interaction during the day

12 or 24 single-use bioreactors in parallel - integrated control of multiple bioreactors offers efficient set-up and control and makes it simple to automate DoE experimental designs

Integrated biological safety cabinet enclosure - for contamination free processing

Automated liquid handler (10 mL and 300 µL pipettes) - for system set-up (media combination and addition), inoculation, bolus feeding and sampling into plates or tubes



## **ambr® 250 Bioreactor Vessels**

**Disposable single-use bioreactors** - no need to clean bioreactors and sensors between runs

**100 - 250 mL working volume** - reduces reagent costs while supporting enhanced off-line analysis using standard techniques

**Integrated pH and DO sensors** - vessel design incorporates disposable sensors to simplify the process of system preparation

**3 step 'Easy Connect' installation** - for all gas, liquid and sensor connections  
 - Step 1 - place vessel in holder and secure with clamp  
 - Step 2 - connect pH probe  
 - Step 3 - Clamp the vessel liquid manifold to the pump feed port

### **Mammalian vessels**

- 100 - 250 mL working volume with baffles
- Dual pitch blade impeller
- Spot based DO sensor
- Disposable pH electrode
- Integrated gas and liquid inlet filters
- Sparge and headspace gassing options
- Robotic compatible cap for sampling
- Integrated exhaust gas condenser



### **Microbial vessels**

- 100 - 250 mL working volume with baffles
- Dual 20 mm Rushton impeller
- Spot based DO sensor
- Disposable pH electrode
- Integrated gas and liquid inlet filters
- Robotic compatible cap for sampling
- Sparge and overlay gassing options
- Integrated condenser for output to exhaust gas analysis (OUR/CER)





## Functions

ambr® 250 automatically controls, feeds and samples 12 or 24 bioreactor/fermenter vessels in parallel

### Bioreactor controller

- Three gases per bioreactor with mass flow sensor:
  - Mammalian
    - $O_2$
    - $CO_2$
    - $N_2$ /air
  - Microbial
    - $O_2$
    - Air
    - $N_2$
- Four positive displacement liquid pumps per bioreactor for high precision at low flow rates
- Individual bioreactor temperature control with heating or cooling
- Individual impeller speed control per bioreactor
- Optional exhaust gas analysis for OUR/CER
- Integrated CIP/SIP for pumps and liquid lines

### Control software

- Fully flexible set up and control interface with simple, dialogue based interaction for quick and easy process creation.
- Fine tuning options including programmable PID control loops and other advanced features
- ambr 250 software is fully integrated with Linmetrics MODDE Design of Experiment (DoE) application, enabling streamlined operation in execution of large DoE studies.

## Applications

ambr® 250 is configurable for microbial fermentation or mammalian cell culture and able to model a wide range of requirements in a variety of applications across biopharm as well as individual biotech.

- R&D and process development for biopharma
- R&D and process development for industrial biotech
- Process robustness experimentation in support of QbD studies
- Automation of DoE approaches to process automation
- Biofuels feedstock preparation R&D



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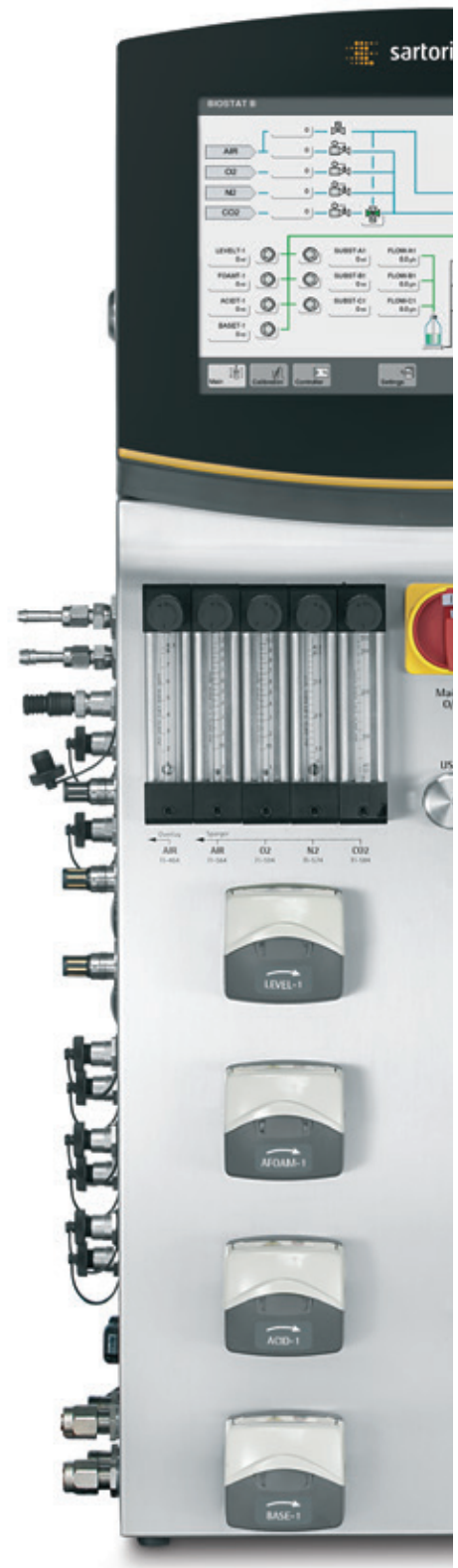
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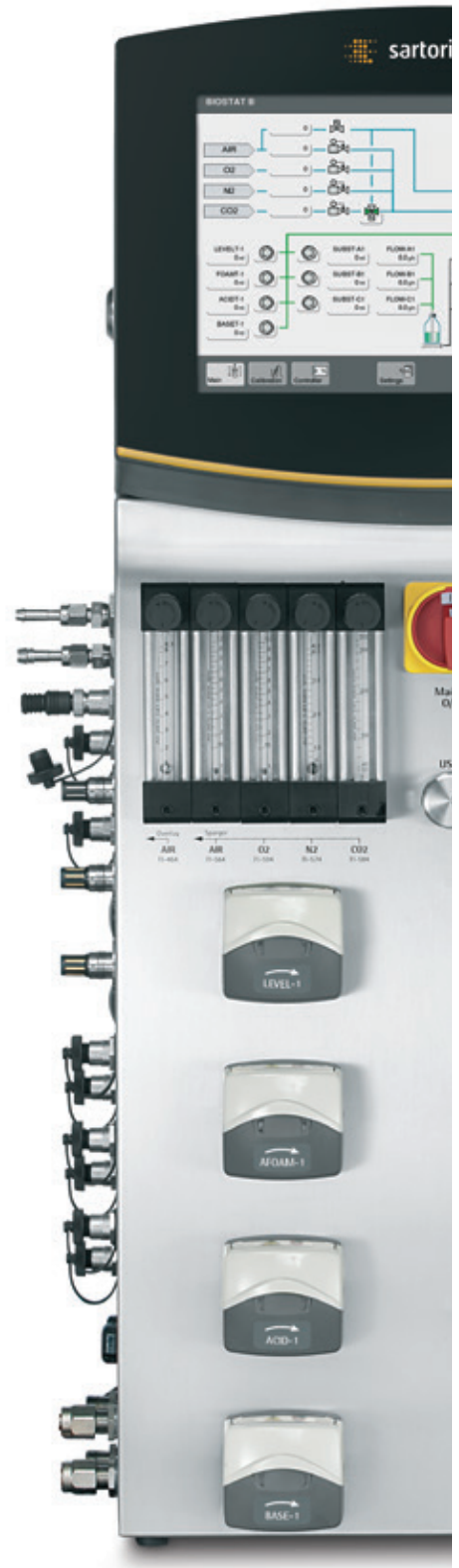
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- Seed and small scale production
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 winner 2013

# RM Rocker

Our proven wave-mixed bioreactor consists of a rocker unit with bag holder and pillow-shaped Flexsafe RM single-use bags for culture volumes from 100 mL to 25 L. Use it as basic system or in combination with the BIOSTAT® B control tower and single-use optical pH and DO sensors to enable advanced control strategies for batch, fed-batch and continuous perfusion.

## Benefits

- Optimized film formula, traceability of raw materials and control of the entire Flexsafe RM bag manufacturing process assure excellent and consistent cell growth of the most sensitive cell lines
- Space-saving, individual control of two RM bags on the same rocker platform
- Easy-to-use rocker with advanced alarms and safety features for reliable cultivation





## 12" Touch Screen

with closed frame; protected against water splashes and dust deposits. Easy-to-use and reliable operation of your BIOSTAT® B due to intuitive design of human-machine-interface and advanced touch-screen technology – even while wearing laboratory gloves.



## New Standards for Interference-Free Measurements

All inlets and ports for, e.g. cooling water, process gasses, electricity, ethernet and potential-free alarm contact are located on the rear panel of the control tower. An equipotential bonding conductor shields the bioreactor against electromagnetic current and ensures interference-free measurements during your processes.



## Control Tower

The control tower contains both the aeration, pump and temperature control modules, saving valuable bench space in your lab.



# BIOSTAT® B

## Configurable Flexibility

Single | Twin Control Tower

One BIOSTAT® B control tower can control up to two vessels completely independently to save valuable lab space. Select culture vessel types and volumes from our range of options, e.g. glass vessel, single-use vessel or wave-mixed Flexsafe® RM bags.



### Fast Load Peristaltic Pumps

Insert the tubing in seconds: Open, insert tube, close, done! No more pinched fingers or torn gloves. Up to four internal pumps can be selected per vessel. Choose between speed-controlled pumps and fixed-speed pumps for feed and corrective agent addition.



### Connections for Sensors and External Accessories

Quick-connect couplings make it easy to attach all cables and supplies to the culture vessels. All connections are clearly marked with the same names used on the rear control tower panel and in the local control software menu for fast, error proof identification.



### Storage Tray

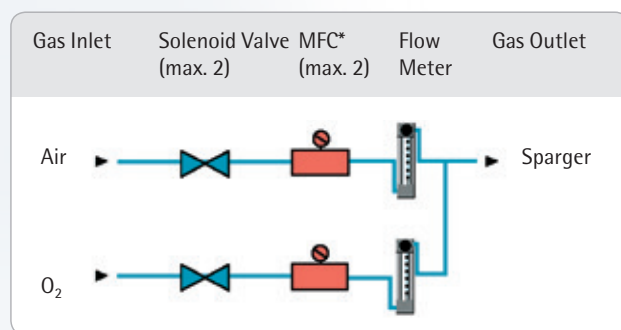
A storage tray for accessories helps you organize your workplace.

# Aeration Strategies

A series of flexible aeration options turns the BIOSTAT® B into a multi-talented bioreactor for a wide range of different applications, including high-cell density microbial fermentation requiring considerable amounts of oxygen to high-cell density cell culture applications requiring removal of excessive carbon dioxide.

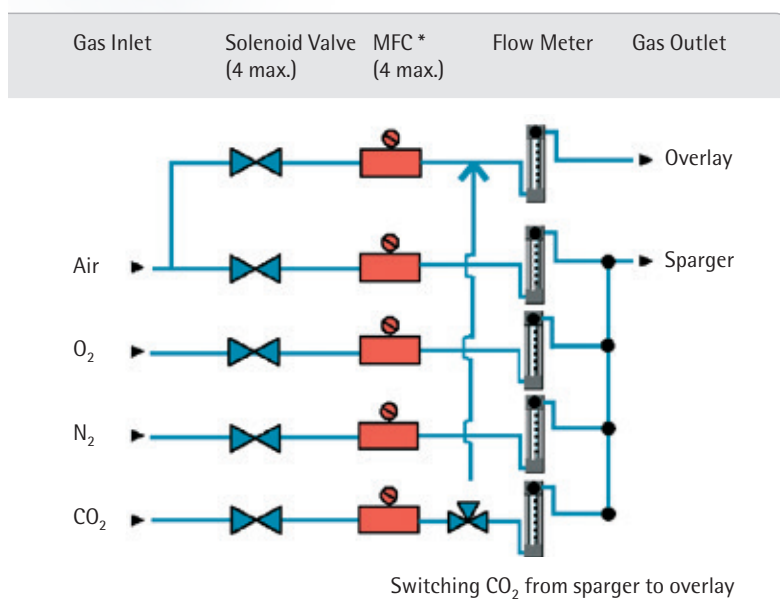
## Microbial Applications in Combination with UniVessel® Glass

Various controller and hardware configurations enable aeration strategies using air or oxygen or classical O<sub>2</sub> enrichment of air. For anaerobic processes, the air inlet can be used for nitrogen. The standard built-in solenoid valves in combination with a flow meter ensure reliable gas supply for simple applications. Optional mass flow controllers provide exact flow rate control of individual gases. In combination with BioPAT® Xgas off-gas analysis, this allows mass balancing consumed and produced gases.



\*MFC = mass flow controller

## Cell Culture and Multi-Purpose Applications in Combination with UniVessel® Glass and UniVessel® SU



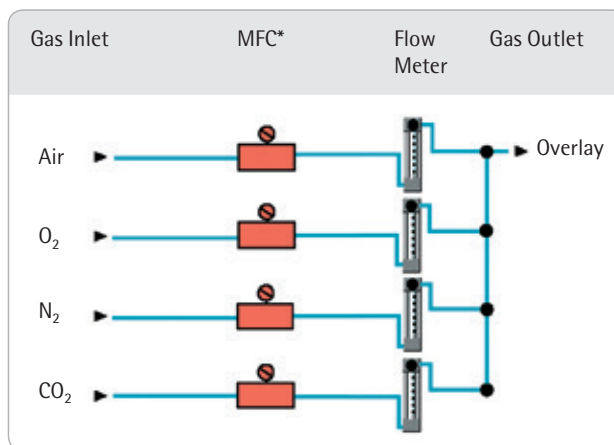
\*MFC = mass flow controller

Five different gas lines, equipped with solenoid valves and/or up to four optional mass flow controllers and flow meters deliver ultimate flexibility and accuracy. The BIOSTAT® B allows you to optionally switch between sparger and overlay supply of CO<sub>2</sub> for pH control. In addition, a constant air | CO<sub>2</sub> mixture can be created in the overlay line to reproduce conditions like those prevailing in a CO<sub>2</sub> incubator.



## Cell Culture Applications in Combination with Our Rocking-Motion Bioreactor

The wave generated in the RM bags due to the rocking motion ensures effective gas exchange through the gas-liquid interface. Four different gas lines for air, O<sub>2</sub>, N<sub>2</sub> and CO<sub>2</sub> are equipped with flow meters and four high-precision mass flow controllers. An integrated pressure sensor continuously measures the pressure inside the bag. To ensure process safety, aeration is switched off when an upper threshold is reached.



\*MFC = mass flow controller

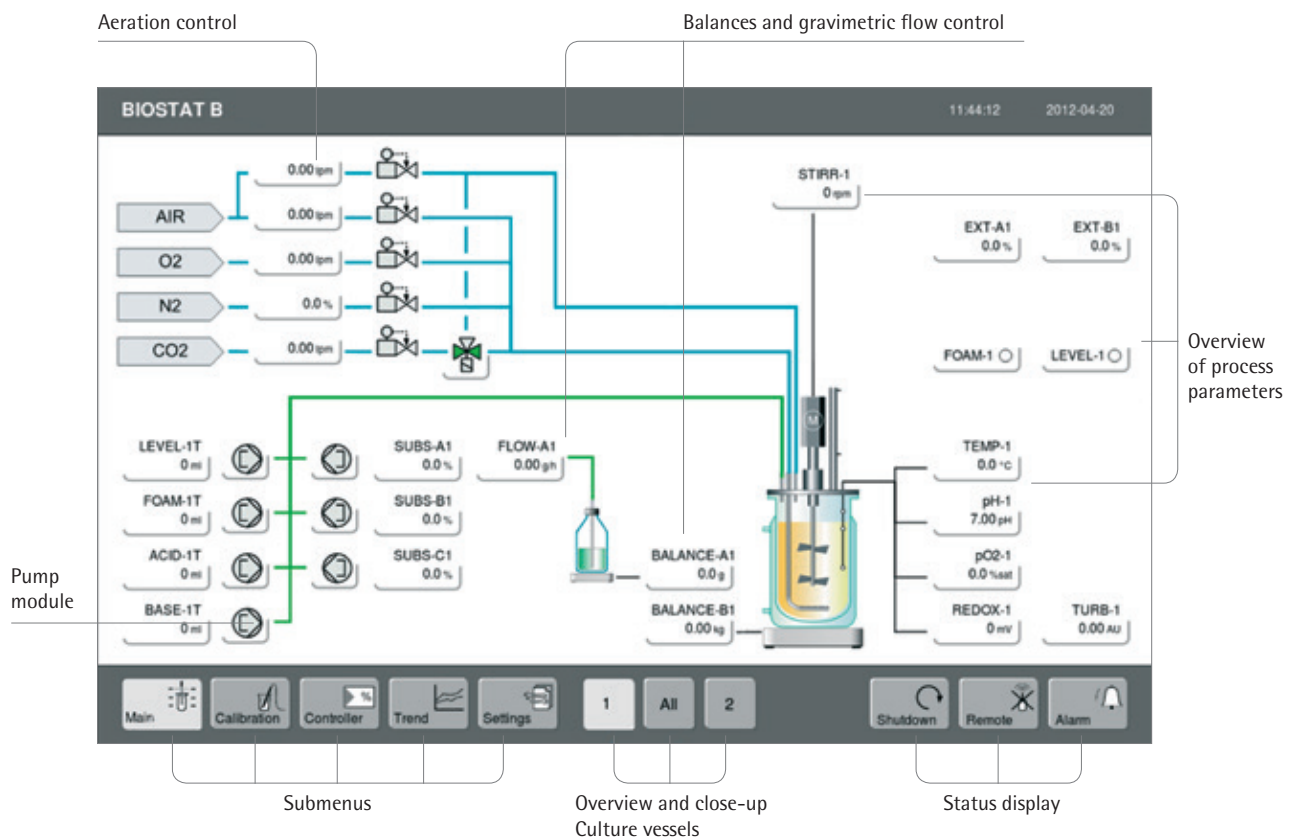
You can configure your BIOSTAT® B aeration system in a similar way as your BIOSTAT® STR single-use stirred tank bioreactor. Get ready for seamless scale-up and -down, from laboratory scale to large scale single-use production.



# DCU – Local Control

Since we introduced the first DCU-controlled bioreactors back in the late 80's, we have installed thousands of such bioreactors to date at leading companies in the pharmaceutical and biotech industry worldwide. We have continuously improved our robust, intuitive-use and industry-proven DCU control technology, now available in its fourth generation. It is our standard local control platform for our BIOSTAT® bioreactors, SARTOFLOW® crossflow filtration units and FlexAct® configurable systems available for a large number of unit operations.

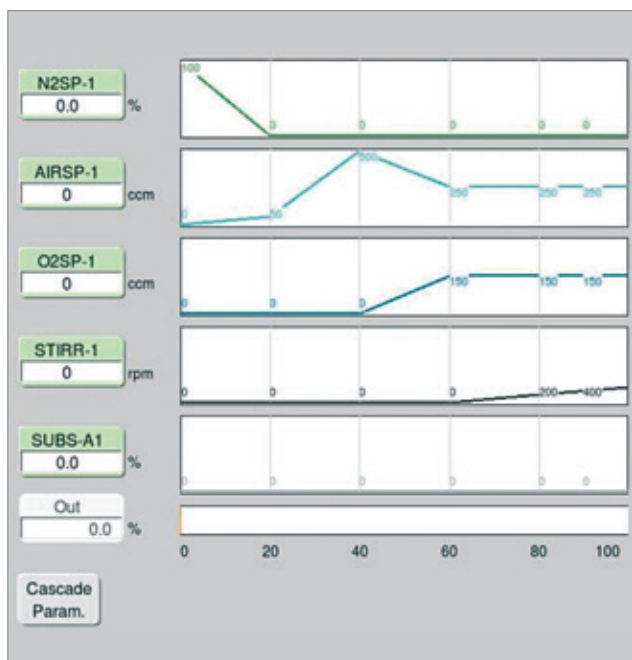
The intuitive touch screen on the control tower is used to locally operate the BIOSTAT® B:





## Automatic Feed Control and Continuous Processing

Design your process strategy or select different options. Configure your BIOSTAT® B with gravimetric feed control, gravimetric level control or substrate addition profiles. This enables you to run your BIOSTAT® B in batch, fed-batch, continuous or perfusion mode.



## Automatic DO Control

Besides classic DO cascade control, we have developed the unique advanced DO controller that gives you more flexibility to develop and optimize your DO control strategy.

The advanced DO controller supports parallel adjustment of all DO affecting parameter settings like stirrer speed and gas flow rates of air and pure oxygen, automatically and simultaneously to control the DO set point. Optionally, you can keep the total gas flow rate constant and the ratio of pure oxygen in the gas mixture is increased automatically to match the oxygen consumption of the culture. This gives you ultimate flexibility to adapt your aeration strategy to your process requirements.

## Automatic pH Control

Control the pH of your culture by automatic acid and base addition or by CO<sub>2</sub> aeration and base addition. If you want to use your BIOSTAT® B for both microbial and cell culture you can configure your bioreactor system with a combined acid control via CO<sub>2</sub> aeration and acid addition.

CO<sub>2</sub>  
Base  
Acid

# BioPAT<sup>®</sup> MFCS – SCADA Process Control

BioPAT<sup>®</sup> MFCS is a multi-functional supervisory control and data acquisition software, specifically developed for bioprocessing applications in upstream and downstream. Every BIOSTAT<sup>®</sup> bioreactor comes with basic data recording functionality of our BioPAT<sup>®</sup> MFCS. Rely on more than 7,000 installations worldwide and over 25 years of experience.

- Multi-user network access for up to 16 process units
- On-line and off-line sample data management, visualization, evaluation
- Remote monitoring, control and alarming



## BioPAT<sup>®</sup> MFCS | win

In addition to providing a genuine SCADA system for conventional and single-use bioprocess applications, the optional advanced version of BioPAT<sup>®</sup> MFCS includes a series of add-on modules:

- Bioprocess optimization with Design of Experiments module (DoE)
- Automation with recipes according to ANSI | ISA-88.01
- 21 CFR, Part 11 compatibility
- Secure integration into company networks
- Flexible device connection facility through OPC
- Expanded data recording and data archiving capabilities
- Alarm recording and transmission to multimedia devices

# Our Mission Is Your Productivity

We are well aware of how important smooth-running equipment is for effective process development and efficient production. That is why we at Sartorius offer you a broad range of preventive technical service, repair and application specialist support. Our ISO certified quality management system ensures that you receive equipment and services that meet the strictest quality requirements. Become one of our many customers who benefit from our local presence worldwide, highly qualified specialists and well-versed technicians.

## Services Offered for BIOSTAT® B

- On-site installation and application specialist support, ensuring that your equipment is perfectly up and unning right from the start
- Factory acceptance and site-acceptance testing
- On-site preventive maintenance and repair
- Consumable and spare part kits



# Technical Specifications

## BIOSTAT® B

Control Tower Weight	
Single   Twin	~ 40   55 kg (88   121 lbs.)
Control Tower Dimensions (W × H × D)	
Single   Twin	410 × 810 × 520 mm (16" × 32" × 20")
Utility Connections	
Power supply	<ul style="list-style-type: none"> <li>– 230 V (± 10%), 50 Hz, max. power consumption 10 A</li> <li>– 120 V (± 10%), 60 Hz, max. power consumption 12 A</li> <li>– Potential equalisation</li> </ul>
International protection rating	IP21
Gases	<ul style="list-style-type: none"> <li>– Gas supply pressure, 1.5 barg</li> <li>– Dry, oil and dust-free</li> <li>– Hose barb for tubing, external dia. = 6 mm</li> </ul>
Water	<ul style="list-style-type: none"> <li>– Water supply pressure, 2 – 8 barg</li> <li>– Flow rate up to 20 lpm</li> <li>– Temperature min. = 4°C</li> <li>– Discharge pressure-less</li> <li>– Hose barb for tubing, external dia. = 10 mm</li> <li>– Degree of hardness: 12 dH max.</li> </ul>
Control Tower	
Housing	Stainless steel, AISI 304
Display	Touch screen, 12", glass, capacitive
Resolution	125 dpi
SCADA communication	Industrial Ethernet
Potential-free (common) alarm contact	•
Safety valve gas pressure	1 bar (14.5 psi) for UniVessel® Glass and SU   100 mbar for RM
Water inlet pressure reduction	1.5 bar (22 psi), integrated pressure control
Motor Drive (Univessel® Glass   SU)	
Maintenance-free, quiet direct drive	Power: 200 W
Maintenance-free top drive with magnetic motor coupling	Power: 200 W
Rotation speed motor, direct coupling	1L: 20 – 2,000 rpm 2L: 20 – 2,000 rpm 5L: 20 – 1,500 rpm 10L: 20 – 800 rpm 2L SU: 20 – 400 rpm
Rocker Platform	
Power supply	<ul style="list-style-type: none"> <li>– 230 V (± 10%), 50 Hz, power consumption 10 A</li> <li>– 120 V (± 10%), 60 Hz, power consumption 12 A</li> </ul>
Power consumption	600 W
International protection rating	IP23
Rocking speed	8 – 42 ± 1 (r/min)
Rocking angle	4 – 10 ± 0.3 (°)

## Process Control | Sensors

Sensor   Measurement Range   Display Accuracy		UniVessel® Glass	UniVessel® SU	Rocking Motion
Temperature	Pt100   0–150°C (temperature control 0–80°C)   0.1°C	•	•	•
Dissolved oxygen, reusable	Polarographic or optical   0–100 %   0.1 %	•	•	
Dissolved oxygen, single-use	Range: 0–100 % Precision: 0.1%		•	•
pH, reusable	Combined measuring electrode Range: 2–12 pH Precision: 0.01 pH	•	•	
pH, single-use	Range: 6.5 – 8.5 pH Precision: 0.1 pH		•	•
Foam control	Electrical conductive sensor, stainless steel, ceramic insulated	•		
Level	Electrical conductive sensor, stainless steel, ceramic insulated	•		
Turbidity	1-channel NIR absorption sensor   0–6 AU   0,01 AU	•		
Redox	Combined measurement with pH sensor   –1,000 – 1,000 mV   1 mV	•	•	
Balance substrate	7 kg max.   1 g 60 kg max.   2 g 300 kg max.   100 g	•	•	•
Balance culture vessel	60 kg max.   2 g	•	•	
RM load cells	30 kg max.   10 g Weight: 9 kg Dimensions: 609   536   60–68 mm 100–240 V VAC   24 VDC   15 W			•
External signal input	0–10 V or 4–20 mA UniVessel® Glass   SU: 4 max. Rocker: 2 max.	•	•	•



## Aeration Module

Outlet to culture vessel   bag	Hose coupling $\varnothing$ external = 6 mm
<b>UniVessel® Glass MO (Microbial)</b>	<b>Two-gas mixing with sparger outlet</b>
AIR with O <sub>2</sub> Enrichment or Gas Flow Ratio mixing along with anaerobic fermentation; for further information, please see page on "aeration strategies"	
Max. total flow	Up to 20 l/min total volume flow
Flow rate controllers	2
Flow rate controller range	Various models available: 0.1–20 liters per min. [lpm] (sparger)
Accuracy of flow rate controllers	± 5 % full scale
Mass flow controllers (optional)	2 max.
Flow rates of mass flow controllers	Various models available: 0.03–20 lpm (sparger)
Accuracy of mass flow controllers	± 1 % full scale
<b>UniVessel® Glass CC (Cell Culture)   UniVessel® SU</b>	<b>Four-gas mixing with sparger and headspace outlet</b>
Additive flow, 4-gas (Air, O <sub>2</sub> , N <sub>2</sub> , CO <sub>2</sub> ) mixture; for further information, please see page on "Aeration Strategies"	
Max. total flow	Up to 13 l/min total volume flow
Number of flow rate controllers	5
Flow rate controller range	Various models available: 3.3 ccm – 1.6 lpm (sparger) 0.16 lpm – 13 lpm (headspace)
Accuracy of flow rate controllers	± 5 % FS
Mass flow controllers (optional)	4 max.
Flow rates of mass flow controllers	Various models available: 1 ccm – 1.5 lpm (sparger) 0.03 lpm – 10 lpm (headspace)
Precision of mass flow controllers	± 1 % FS
<b>UniVessel® Glass, Dual Use</b>	<b>Four-gas mixing with sparger and headspace outlet</b>
Additive flow, 4-gas (Air, O <sub>2</sub> , N <sub>2</sub> , CO <sub>2</sub> ) mixture; for further information, please see page on "Aeration Strategies"	
Max. total flow	Up to 20 l/min total volume flow
Flow rate controllers	5
Flow rate controller range	Various models available: 3.3 ccm – 20 lpm (sparger) 50 ccm – 20 lpm (headspace)
Accuracy of flow rate controllers	± 5 % full scale
Mass flow controllers (optional)	4 max.
Flow rates of mass flow controllers	Various models available: 0.6 ccm – 20 lpm (sparger) 10 ccm – 20 lpm (headspace)
Precision of mass flow controllers	± 1 % FS
<b>Rocker</b>	<b>Four-gas mixing with sparger and headspace outlet</b>
Additive flow, 4-gas (Air, O <sub>2</sub> , N <sub>2</sub> , CO <sub>2</sub> ) mixture; for further information, please see page on "Aeration Strategies"	
Max. total flow	Up to 10 l/min total volume flow
Max. CO <sub>2</sub> flow	One bag: 1 × 1 lpm Twin bag: 2 × 0.5 lpm
CO <sub>2</sub> concentration controller	0.8 % – 15 %   ± 5 %
Flow rate controllers	4
Flow rate controller range	Various models available: 16 ccm – 36 lpm (headspace)
Accuracy of flow rate controllers	± 5 % full scale
Mass flow controllers (optional)	2 max.
Flow rates of mass flow controllers	Various models available: 3 ccm – 3 lpm (headspace)
Precision of mass flow controllers	± 1 % full scale

## Pump Module

Built-in Pumps	
Fixed speed (on   off controlled)	Watson Marlow 114, Fast Load pump head
– Speed 5 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0–0.1 ml/min ID: 0.8 mm: 0.05–2.4 ml/min ID: 1.6 mm: 0.01–0.7 ml/min ID: 2.4 mm: 0.03–1.5 ml/min ID: 3.2 mm: 0.05–2.4 ml/min ID: 4.8 mm: 0.09–4.3 ml/min
– Speed 44 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0.02–0.9 ml/min ID: 0.8 mm: 0.04–1.8 ml/min ID: 1.6 mm: 0.12–6.2 ml/min ID: 2.4 mm: 0.26–12.8 ml/min ID: 3.2 mm: 0.41–20.7 ml/min ID: 4.8 mm: 0.75–37.4 ml/min
Speed-controlled	Watson Marlow 114, Fast Load pump head
– Speed 0.15–5 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0–0.1 ml/min ID: 0.8 mm: 0.01–0.2 ml/min ID: 1.6 mm: 0.02–0.7 ml/min ID: 2.4 mm: 0.04–1.5 ml/min ID: 3.2 mm: 0.07–2.4 ml/min ID: 4.8 mm: 0.13–4.3 ml/min
– Speed 5–150 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0.1–3 ml/min ID: 0.8 mm: 0.2–6 ml/min ID: 1.6 mm: 0.7–21 ml/min ID: 2.4 mm: 1.45–43.5 ml/min ID: 3.2 mm: 2.35–70.5 ml/min ID: 4.8 mm: 4.25–127.5 ml/min
External Pumps	
Speed-controlled	Watson Marlow 120, Fast Load pump head, up to 200 rpm

## Temperature Control Module

Heating and Cooling	Heating Only
For UniVessel® Glass Single-wall Culture Vessels	
Electrical heating system and automatic cooling water valve; connection to heating blanket and cooling finger	–
Temperature control of 8°C above cooling-water inlet temperature up to 60°C	–
Heating blanket capacities 1 L   2 L   5 L   10 L: 100   170   400   780 W	–
For UniVessel® Glass Double-wall (Jacketed) Culture Vessels	
Open thermostat system with recirculation pump and automatic cooling water valve	–
Temperature control of 8°C above cooling-water inlet temperature of up to 80°C	–
Heating capacity: 600 W	–
For UniVessel® SU Single-use Culture Vessels	
Open thermostat system with recirculation pump and automatic cooling water valve; connection to heating   cooling jacket	Electrical heating blanket
Temperature control up to 50°C	Temperature control up to 50°C Heating capacity 2L: 200 W
For BIOSTAT® RM Rocking-motion Bioreactor	
Open thermostat system with recirculation pump and automatic cooling water valve	Electrical heating plates
Temperature control of 8°C above cooling-water inlet temperature of up to 40°C	Temperature control from room temperature up to 40°C
Heating capacity: 600 W	Heating plates: 2 × 140 W



### Culture Vessel UniVessel® Glass

Autoclavable Single-wall or Double-wall (Jacketed) Glass Vessel	1 L	2 L	5 L	10 L
Material	Borosilicate glass, stainless steel AISI 316L, EPDM			
Sizes [L]	1	2	5	10
Total volume [L]	1.6	3	6.6	13
Working volume [L]	0.4–1	0.6–2	0.6–5	1.5   5–10
Top port 19 mm   12 mm   6 mm	3   2   6	3   2   9	3   3   8	5   2   9
<b>UniVessel®, Single-wall</b>	<b>1 L</b>	<b>2 L</b>	<b>5 L</b>	<b>10 L</b>
Weight <sup>1</sup> [kg]	6	7	12	19
Space requirements in autoclave [W × H × D mm]	200 × 540 × 270	230 × 600 × 300	260 × 730 × 340	330 × 860 × 420
Space requirements in autoclave, reduced <sup>2</sup> [W × H × D mm]		480 × 410 × 300	530 × 510 × 410	540 × 630 × 420
<b>UniVessel®, Double-wall</b>	<b>1 L</b>	<b>2 L</b>	<b>5 L</b>	<b>10 L</b>
Weight <sup>1</sup> [kg]	7	8	14	23
Space requirements in autoclave [W × H × D mm]	330 × 540 × 270	380 × 600 × 300	410 × 730 × 340	450 × 860 × 420
Space requirements in autoclave, reduced <sup>2</sup> [W × H × D mm]		480 × 410 × 300	530 × 510 × 410	540 × 630 × 420

<sup>1</sup> With tripod and head plate, without medium

<sup>2</sup> Adapter for exhaust cooler to reduce height in the autoclave optionally available

### Culture Vessel UniVessel® SU

Single-use vessel made of pre-sterilized polycarbonate for cell culture applications	
Total volume [L]	2.6
Working volume [L]	0.6–2
Max. temperature	50 °C
Operating pressure	< 0.5 barg





# Basic Configurations

The BIOSTAT® B is a highly flexible and modular system that can be individually adapted to the requirements of your application. Below you will find an overview of the basic equipment which you can extend with diverse options. Please contact your Sartorius Stedim Field Representative or Application Specialist for additional information.

## Microbial Packages for UniVessel® Glass

Volume
Single
Twin
Digital controller, color display with touch screen
Control temperature, pH, DO, stirrer speed
Maintenance-free, quiet motor
Storage tray for accessories
Aeration module with 2 solenoid valves
2 flow rate controllers for manual flow rate control (AIR   N <sub>2</sub> , O <sub>2</sub> )
Software configured for microbial applications
4-stage DO cascade
BioPAT® MFCS software for data acquisition
2 integrated pumps for pH control (acid   base)
Temperature control module for double-wall vessels
2 external signals 0 – 10 V
Standard test and documentation
Installation set for the gas and water connections
Power cable
Double-wall culture vessel
Stirrer shaft with single-mechanical seal and direct coupling
2 addition bottles for correction agents
Exhaust cooler
Aeration filters
Rushton impellers
Ring sparger
4-way addition port
Inoculation port
Harvest pipe, height-adjustable
Manual sampler
Tool set for disassembly of vessel
Pt100 temperature sensor
pH sensor
DO sensor, amperometric

## Cell Culture Packages for UniVessel® Glass

Volume
Single
Twin
Digital controller, color display with touch screen
Control temperature, pH, DO, stirrer speed
Maintenance-free, quiet motor
Storage tray for accessories
Aeration module with 4 solenoid valves
5 flow rate controllers for manual flow rate control (AIR Overlay, AIR sparger, N <sub>2</sub> , O <sub>2</sub> , CO <sub>2</sub> )
Software configured for cell culture applications
4-stage DO cascade
BioPAT® MFCS software for data acquisition
1 integrated pump for pH control (base)
Temperature control module for single-wall vessels
2 external signals 0–10 V
Standard test and documentation
Installation set for the gas and water connections
Power cable
Single-wall culture vessel with tripod
Heating blanket 120   230 V
Stirrer shaft with single mechanical seal and direct coupling
1 addition bottle for correction agent
Exhaust cooler
Aeration filters
3-blade segment impeller
Microsparger
4-way addition port
Harvest pipe, height-adjustable
Manual sampler
Tool set for vessel dismantling
Pt100 temperature sensor
pH sensor
DO sensor, amperometric

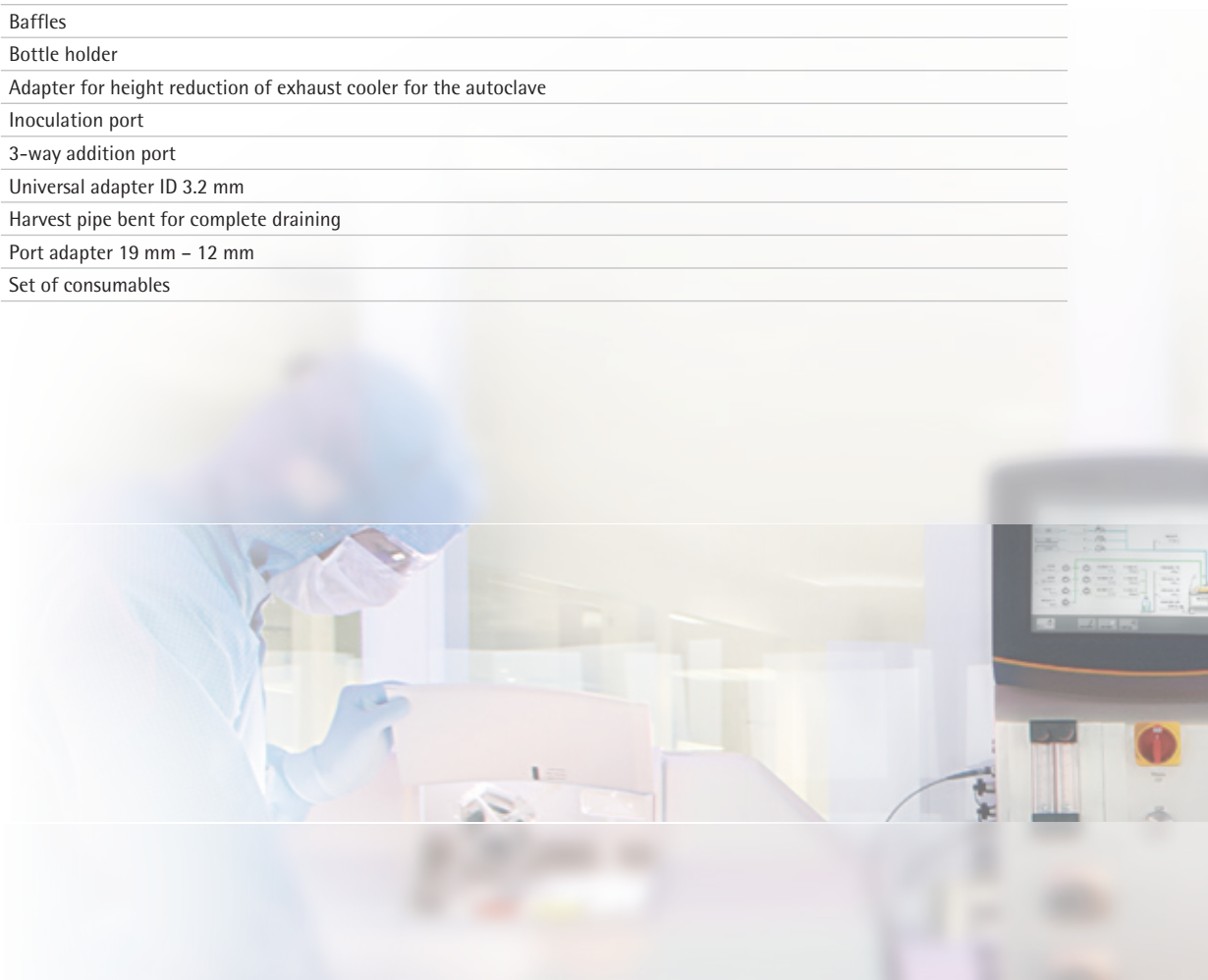
# Options

## Control System

Advanced DO controller
Flexible switching CO <sub>2</sub> from sparger to headspace
Software for dual use MO   CC
Mass flow controller
Antifoam control via sensor
Mechanical foam destroyer (MO applications)
Level control via sensor
Weight measurement substrates   culture vessel
Gravimetric feed   level control
Substrate addition via time profile
Redox (ORP) measurement
Turbidity measurement
BioPAT® Xgas O <sub>2</sub>   CO <sub>2</sub> off-gas analysis
System IQ   OQ
BioPAT® MFCS SCADA system

## UniVessel® Glass

Double-wall culture vessel
Magnetic coupling for drive motor
STT connectors for safe inoculation and media transfer
Cooling finger (single-wall culture vessels)
Spin filter for perfusion mode
Aeration basket for organisms sensitive to shear stress
Rushton impellers   3-blade segment impellers
Baffles
Bottle holder
Adapter for height reduction of exhaust cooler for the autoclave
Inoculation port
3-way addition port
Universal adapter ID 3.2 mm
Harvest pipe bent for complete draining
Port adapter 19 mm – 12 mm
Set of consumables



# Sales and Service Contacts

For further contacts, visit [www.sartorius-stedim.com](http://www.sartorius-stedim.com)

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sartorius stedim  
biotech

## BIOSTAT® D-DCU Your "Fast Lane" to Production



turning science into solutions

# BIOSTAT® D-DCU

## Your "Fast Lane" to Production

The BIOSTAT® D-DCU is a compact bioprocess system available in microbial or cell culture versions with vessel choices from 10 to 200L working volume.

The optimized and proven design of the BIOSTAT® D-DCU is the result of thorough analysis of the most required features and functions from over thirty years of stainless steel fermenter | bioreactor design experience. This standardized solution eliminates design times, allows faster delivery, reduces cost, guarantees trouble free operation, allows for global service support as well as spare part availability.

The BIOSTAT® D-DCU incorporates many desirable and advanced features to fulfill virtually any demand for modern bioprocess application, such as: Automatic Sterilization in Place (SIP), Cleaning in Place (CIP), dual pH and DO measurement capability, lid lifting device, Water Intrusion Test (WIT)-Ready filter housings, dual exhaust filter housing line, tube and shell exhaust cooler, exhaust heater, automatic or manual addition arrays as well as other accessories. Furthermore, the BIOSTAT® D-DCU is designed to interface single-use storage bags for media addition and harvest as well as the TAKEONE® aseptic sampling system. The modular approach allows multiple configurations (from baseline to fully featured) to meet every need and budget.

### Three subsystems comprise every BIOSTAT® D-DCU:

- Control tower with integrated gas mixing and pump module
- Culture vessel with bottom agitation system
- Supply Unit, open frame skid and compact stainless steel piping module

The control tower features best-in class control capabilities utilizing proven industrial hardware. It is operated via a simple and intuitive 19" touch screen which keeps staff training to a minimum. The compact design of the stainless steel housing reduces the footprint and saves precious space.

The jacketed stainless steel culture vessel, with spiral baffles for efficient and homogenous heat transfer, are available in 3:1 or 2:1 aspect height to diameter ratio. The gear free bottom drive agitation system provides long-term operation at minimal noise. The zero dead volume sanitary radial diaphragm harvest and radial type sampling valves provide fresh samples without residual pockets and are easy to clean and maintain.

The supply unit includes all process piping for temperature control as well as the exhaust and gas inlet lines. Due to the open frame design direct access for operation and easy maintenance is ensured. Furthermore, minimal floor contact points allow easy cleaning even underneath the skid.

An extended documentation and qualification package is available to support regulatory requirements.

The BIOSTAT® D-DCU is available in both Single and Twin controller configuration. It increases flexibility and allows control of two separate processes at the same time – even with different size culture vessels – but independently from each other.







## Features

- Single or Twin Configurations
- Available in incremental sizes from 10 to 200 L
- Preconfigured systems or choose from an extensive list of options
- Powerful industrial rated DCU control system with 19" TFT color touch screen
- Automatic Sterilization in Place (SIP) included
- Automatic Cleaning in Place (CIP) optional
- Designed to interface single-use bags and sampling systems including the new TAKEONE® aseptic sampling system
- Measurement and control opportunities of pH, DO, temperature, foam, level, vessel pressure, vessel weight, substrate addition, gas mixing, agitation, gravimetric feed and harvest control, constant total gas flow control, redox and turbidity, weight of storage vessels etc.
- Up to six integrated peristaltic pumps per vessel with options for fixed or variable speed control
- Choice of polarographic or optical DO sensors
- Superior gas mixing with up to six flow meters and mass flow controllers
- Extended documentation package available, including logbook and 3-Level password protection
- Minimal floor contact points for ease of cleaning
- Global spare part and service availability

# Configure a System Utilizing Options (Like Gasmix, CIP) from a Baseline Unit

## Supply Unit

The Supply Unit includes all process piping for temperature control as well as the exhaust and gas inlet lines. The open piping frame and ergonomic design of the skid allows for good and direct access to valves filter housings etc. All sanitary piping is sloped | self drained. The Supply Unit for culture vessels with 10 – 30 L offers a choice of lockable casters or leveling | support feet. The Supply Unit for culture vessel with 50 – 200 L is equipped with leveling | support feet. Furthermore, the Supply Units can be separated in two pieces allowing easy movement to the site of installation.

## Culture vessel

Available culture vessels from 10 L, 20 L, 30 L, 50 L, 100 L to 200 L working volume, with a total volume aspect ratio of (H:D) 2:1 or 3:1.

## Sterilization in Place (SIP)

For ease of operation, automatic sterilization of the culture vessel, gas inlet and exhaust gas flow path are included. Addition groups, sampling valves and drain valves are either manually or automatically sterilized.

## **NEW** Cleaning in Place (CIP)

The BIOSTAT® D-DCU offers state of the art CIP solutions with integrated SIP | CIP headers and now also with an optional mobile CIP cart or the ability to connect to 3rd party CIP systems providing an electronic handshake between the control systems. Integrated CIP features allow the operator to effectively, reproducibly and automatically clean the complete system including the culture vessel, gas inlets, exhaust lines, addition lines and transfer groups.

## **NEW** Single-use sampling

The TAKEONE® aseptic sampling system is single-use and delivered ready to use. While traditional sampling devices require cleaning, preparation and sterilization after each use, the TAKEONE® single-use sampling system saves valuable time by being fully disposable.



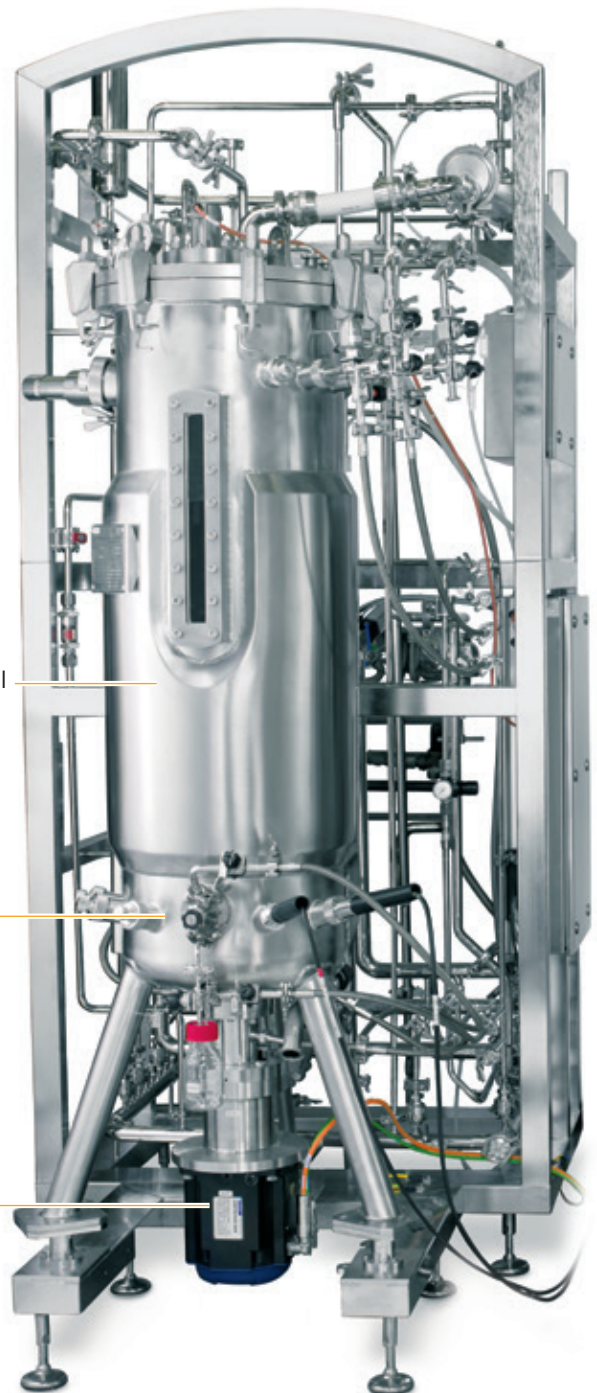
## Agitation

Bottom drive agitation is available with a double mechanical seal. The high performance servo drive motor assembly combines low shear, gentle agitation for cell cultures and high speed mixing for microbial high cell density cultivation, ensuring high oxygen transfer rates. The motor is gear-free for quiet operation – even at high speed ranges.

## Supply Unit

Culture Vessel

Agitation





## Control Tower

### Control Tower

The Control Tower is available in single or twin configurations. The integrated DCU control system belongs to the most proven and advanced bioprocess controllers ever developed. Utilizing proven technology and expert engineering, our existing in-house systems bring powerful control capabilities to the sophisticated biotechnology market. Proven industrial control hardware ensures reliable system performance.

The DCU can be easily expanded and reconfigured to meet evolving research or process requirements, including scale-up from laboratory fermenters or scale-down to mimic production process conditions.

For data logging, the Digital Control Unit (DCU) includes a PC interface for SCADA software connection (for example BioPAT® MFCS). DCU OPC communication software is available for interface to other OPC compliant SCADA and DCS packages. Using a local controller for local process control in combination with a high level SCADA system ensures process control safety.

#### Intuitive Touchscreen

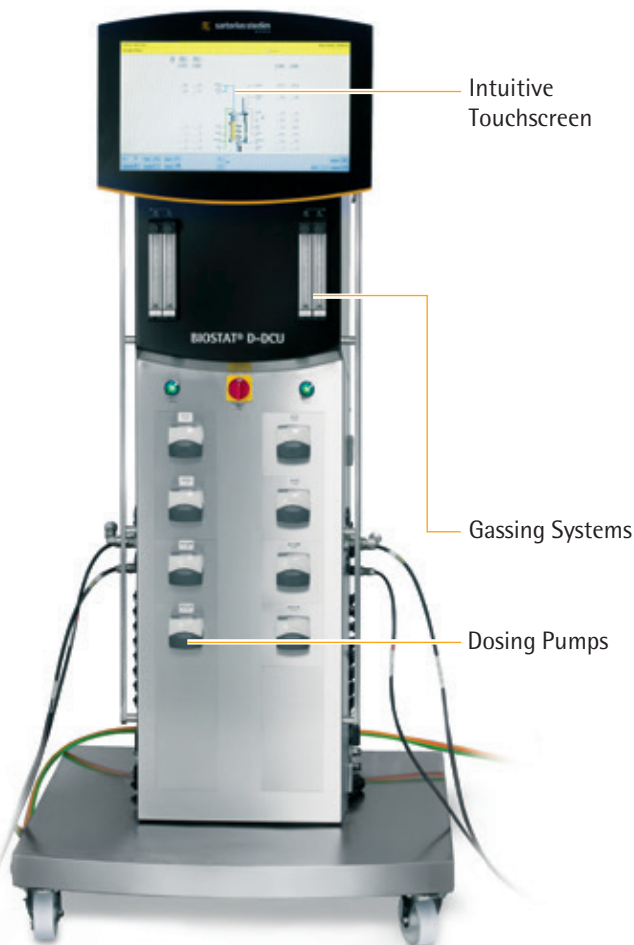
The control system presents an "intuitive-to-use", large 19" TFT color touch screen for excellent local operation and process control for each culture vessel. Clearly designed screens provide an excellent process value overview and operation.

#### Gassing Systems

A variety of spargers are available for microbial and cell culture use. All systems provide individual flow rates and gas blending for each culture vessel. Gas flow rates are adjustable via precision flow meters with optional thermal mass flow controllers available for each flow path (each gas).

#### Dosing Pumps

Up to six integrated fast load peristaltic pumps per vessel may be chosen for addition of corrective agents, feeding, as well as culture volume control. Up to four of the six can be analog speed controlled pumps. Several ranges are available for both fixed and speed controlled pumps. Additionally, external pumps for feeding can be easily connected.

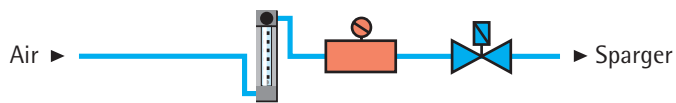


# BIOSTAT® D-DCU

## Gassing Strategies

### Airflow

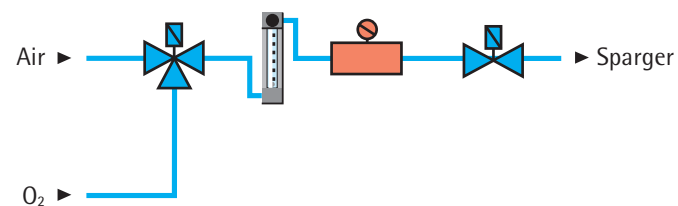
Utilizing one flow path for air, a flow meter visually indicates and controls the sparger flow rate. An optional mass flow controller may be integrated to control and measure the flow range via manual adjustment or automatically in conjunction with the DO controller.



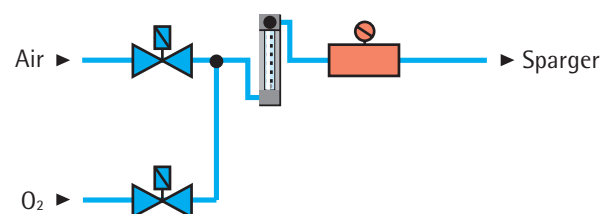
### O<sub>2</sub>-Enrichment





Utilizing two flow paths for Air and O<sub>2</sub> flows, the flow meters visually indicate and allow manual adjustments of the sparger flow rate. O<sub>2</sub> is pulsed via solenoid valve, flowing only when required to maintain the dissolved oxygen (DO) setpoint. Air is not provided at this time. A mass flow controller can be integrated to measure and control the total gas flow range via manual adjustment or automatically in conjunction with the DO controller.

#### O<sub>2</sub>-Enrichment, design up to 50 L/min



#### O<sub>2</sub>-Enrichment, design up to 300 L/min

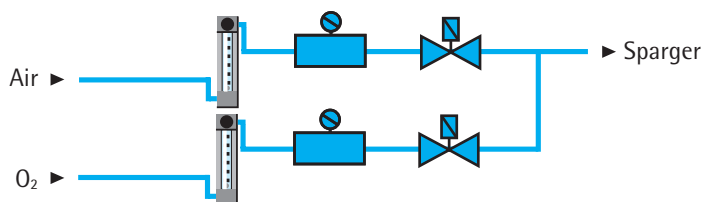


-  Flow meter
-  Optional Gas switch
-  Dosing shut-off valve
-  Optional dosing shut-off valve



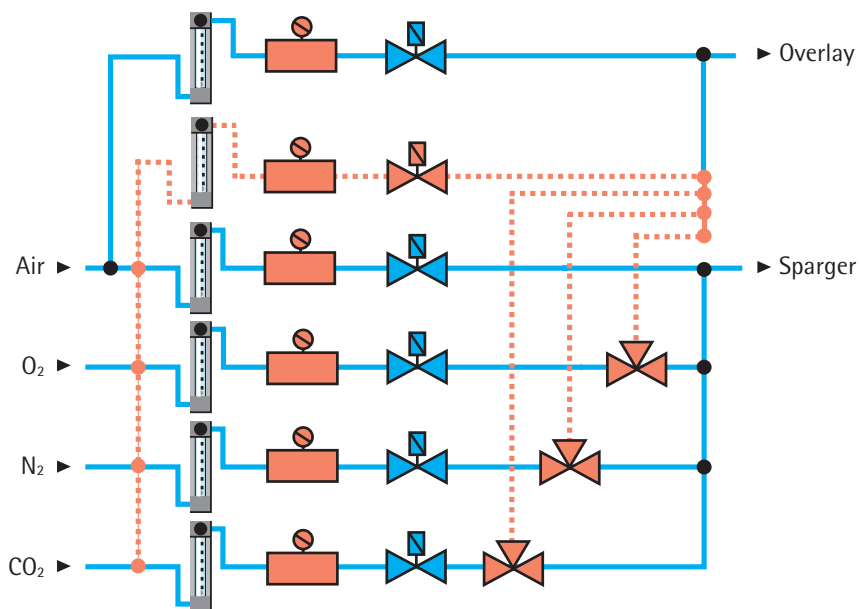
## Gas Flow Ratio

Utilizes two flow paths with mass flow control valves for Air and  $O_2$  flow. Flow meters visually indicate the flow of Air and  $O_2$ . Both mass flow controllers can be operated manually or automatically in conjunction with the DO controller.



## Advanced Additive Flow

Allows up to six gas flow paths. Solenoid valves select air,  $O_2$ ,  $N_2$  and  $CO_2$  for simultaneous flow to the sparger and air to overlay. Up to six flow meters visually indicate and set the flow rate for each gas. One additional gas flow path can be added to sparger or overlay outlet. Furthermore, two 3-way solenoid valves can be installed to switch the dedicated gas from sparger to overlay (incl. soft switch). The design does support the installation of up to six mass flow controllers, which makes constant sparger gas flow control as well as constant overlay gas flow control possible.



# BioPAT® DCU – Automation Solutions for Advanced Process Control and Documentation

Our DCU (digital control unit) controller belongs to the most proven, reliable and advanced bioprocess controllers ever developed. Use of a modular system design has enabled us to offer a broad range of flexible and cost-effective solutions for reusable and single-use systems from R&D to production. DCU control systems are specially tailored for fermentation, cell culture and downstream processing like cross flow filtration applications.

DCU control systems allow for independent and simultaneous operation of multiple processes.

## Discover the potential of our standard software...

- Superior process value overview
- Sensor calibration
- In-process recalibration
- Alarm monitoring
- Trend display
- Automatic Sterilization in Place

## ... and profit from advanced features

- Overview of all vessels or single vessel display
- Controller status indication
- Single or group calibration
- Advanced DO controller
- Gravimetric flow control for very precise feeding
- Gravimetric harvest control
- Constant total gas flow control
- Automatic Cleaning in Place



# BioPAT® MFCS – The Bioprocess SCADA System

BioPAT® MFCS is our SCADA software for supervisory bioprocess control and data acquisition. Provides GMP compliant documentation of your valuable process data and ensures reliable process control in combination with the advanced BIOSTAT® D-DCU for local process control. Supplied with every BIOSTAT® D-DCU package, the new BioPAT® MFCS is ideal for efficient data acquisition and trend monitoring.

**The optional, advanced version of BioPAT® MFCS includes modules such as:**

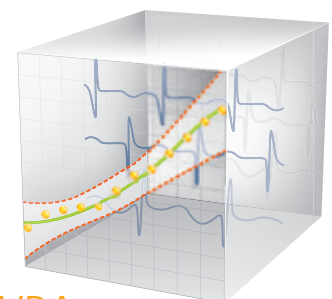
- Multi-user network access for up to 16 process units
- Automation with recipes according to ANSI | ISA 88.01
- 21 CFR, Part 11 compatibility
- Multivariate Data Analysis modules



◀ Learn more about the new BioPAT® MFCS:  
[www.sartorius-stedim.com/biopatmfcs](http://www.sartorius-stedim.com/biopatmfcs)

## BioPAT® SIMCA-Online for Continuous Real-time Quality Control

Continuous real-time quality control and assurance is highly desired in biopharmaceutical manufacturing. Unique on the market and developed according to GAMP 5, BioPAT® SIMCA-online is your software solution for real-time multivariate statistical process monitoring and control. The software permits early detection of process deviations. It provides user guidance to simplify root-cause analysis by displaying easy-to-understand graphics.



**MVDA**  
Online Multivariate  
Data Analysis

# BIOSTAT® D-DCU MO – Package Overview: O<sub>2</sub>-Enrichment

Package overview	10 L	20 L	30 L	50 L	100 L	200 L
Cat. No. 208 VAC   Culture vessel H:D ratio	RDD-M10L OBRES3   2:1	RDD-M20L OBRES3   2:1	RDD-M30L OBRES3   2:1	RDD-M50L OBRES3   2:1	RDD-M1HL OBRES3   2:1	RDD-M2HL OBRES3   2:1
Cat. No. 400 VAC   Culture vessel H:D ratio	RDD-M10L OBRET4   3:1	RDD-M20L OBRET4   3:1	RDD-M30L OBRET4   3:1	RDD-M50L OBRET4   3:1	RDD-M1HL OBRET4   3:1	RDD-M2HL OBRET4   3:1
Control Unit	Twin configuration optional					
Digital controller, color display with touch screen	•					
Control capabilities						
Temperature, pH, DO (Multi stage cascade), Stirrer speed	•					
Substrate A and Substrate B	•					
Foam via conductive sensor	•					
High foam alarm	•					
Automatic full vessel sterilization sequence	•					
Gear and maintenance free agitation motor	•					
Gassing strategy	O <sub>2</sub> -Enrichment (Airflow and Gas Flow Ratio optional)					
Flow meter	•					
Solenoid valve for oxygen enrichment	• (Mass Flow Controller optional)					
Peristaltic pumps (integrated)	3 for Acid   Base   Antifoam unused pump can be configured as substrate pump (Up to 6 pumps per site)					
Supervisory Process Control Software						
BioPAT® MFCS	•					
Supply frame	Open frame design					
Temperature control system	Closed loop system with recirculation pump and heat exchanger for heating and cooling					
Piping with valves and steam traps for automatic in-situ sterilization	•					
Culture Vessel	Jacketed stainless steel vessel with vertical sight glass and bottom agitation					
Stirrer shaft with Double Mechanical Seal (DMS)	•					
Condensate pressurization of buffer system DMS steam   compressed air	•   0					
6-blade disk impeller	3					
Stainless steel filter housing for air Inlet and exhaust filter incl. filter cartridges	•					
Pressure gauge –1   3 barg	•					
Aeration tube with Ring sparger	•					
Exhaust cooler	•					
4-Baffles (removable)	•					
Resterilizable sampling valve	•					
1-Channel Sacova valve for needle free additions	•					
3-Channel Sacova valve for needle free additions	•					
Lamp for vessel illumination	•					
Storage bottles	3					
Bottom harvest valve	•					
pH Electrode, cable	•					
DO Electrode, cable	•					
Pressure sensor, cable	•					
Foam sensor, cable	•					
Temperature sensor Pt 100	•					
High-foam sensor with installation adaptor, cable	•					
Options						
Mobile CIP unit with DCU interface	0					
Culture vessel weight measurement   control	0   0					
Automatic vessel pressure control	0					
Pressure hold test	0					
Lid lifting device 10-20 L   30-200 L	--   0					
Dual pH measurement   Dual DO measurement	0   0					
Containment sampling system	0					
WIT ready filter housings for Inlet and Exhaust filter	0					
Exhaust heater   Dual Exhaust filter line (parallel)	0   0					
Temperature measurement of condensate trap	0					
Cleaning in Place (CIP)	0					
Resterilizable 4-valve addition array manual   automatic	0   0					
Automatic harvest valve	0					
Transfer group	0					
Speed controlled pumps for feeding	0					

Broad range of accessories available. Please contact us for further details.

• = included, -- = unavailable, 0 = option



# BIOSTAT<sup>®</sup> D-DCU CC – Package Overview:

## Advanced Additive Flow

Package overview	10 L	20 L	30 L	50 L	100 L	200 L
Cat. No. 208 VAC   Culture vessel H:D ratio	RDD-C10L ABRES3   2:1	RDD-C20L ABRES3   2:1	RDD-C30L ABRES3   2:1	RDD-C50L ABRES3   2:1	RDD-C1HL ABRES3   2:1	RDD-C2HL ABRES3   2:1
Cat. No. 400 VAC   Culture vessel H:D ratio	RDD-C10L ABRES4   2:1	RDD-C20L ABRES4   2:1	RDD-C30L ABRES4   2:1	RDD-C50L ABRES4   2:1	RDD-C1HL ABRES4   2:1	RDD-C2HL ABRES4   2:1
<b>Control Unit</b>	<b>Twin configuration optional</b>					
Digital controller, color display with touch screen	•					
Control capabilities						
Temperature, pH, DO (Multi stage cascade), stirrer speed	•					
Substrate A – D	•					
Foam via conductive sensor	•					
High Foam alarm	•					
Automatic full and empty vessel sterilization sequence	•					
Gear and maintenance free agitation motor	•					
Gassing strategy	Advanced Additive Flow					
Flow meter sparger	• for Air, O <sub>2</sub> N <sub>2</sub> , CO <sub>2</sub>					
Flow meter overlay	• for Air					
Automatic Gassing strategy of Air, O <sub>2</sub> , N <sub>2</sub> , CO <sub>2</sub> for sparger	• via solenoid valves (Mass Flow Controller optional)					
Peristaltic pumps (integrated)	2 for Base   Afoam unused pump can be configured as substrate pump (Up to 6 pumps per system)					
<b>Data acquisition and trend monitoring software</b>						
BioPAT <sup>®</sup> MFCS	•					
<b>Supply frame</b>	<b>Open frame design</b>					
Temperature control system	Closed loop system with recirculation pump and heat exchanger for heating and cooling					
Solenoid valves and steam traps automatic in-situ sterilization	•					
<b>Culture Vessel</b>	<b>Jacketed stainless steel vessel with vertical sight glass and bottom agitation</b>					
Stirrer shaft with Double Mechanical Seal (DMS)	•					
Condensate pressurization of buffer system DMS steam   compressed air	•   0					
3-blade segment impeller	2					
Stainless steel filter housing for Air Inlet (Sparger and Overlay) and Exhaust filter incl. filter cartridges	•					
Pressure gauge –1   3 barg	•					
Aeration tube with micro sparger	•					
Exhaust Cooler	•					
4-Baffles (removable)	•					
Resterilizable sampling valve	•					
1-Channel Sacova valve for needle free additions	•					
3-Channel Sacova valve for needle free additions	•					
Lamp for vessel illumination	•					
Storage bottles	2					
Bottom harvest valve	•					
pH Electrode, cable	•					
DO Electrode, cable	•					
Pressure sensor, cable	•					
Foam sensor, cable	•					
Temperature sensor Pt 100	•					
High-foam sensor with installation adaptor, cable	•					
<b>Options</b>						
Mobile CIP unit with DCU interface	0					
Culture vessel weight measurement   control	0   0					
Automatic vessel pressure control	0					
Pressure hold test	0					
Lid lifting device 10-20 L   30-200 L	--   0					
Dual pH measurement   Dual DO measurement	0   0					
Containment sampling system	0					
WIT ready filter housings for Inlet and Exhaust filter	0					
Exhaust heater   Dual Exhaust filter line (parallel)	0   0					
Temperature measurement of condensate trap	0					
Cleaning in Place (CIP)	0					
Resterilizable 4-valve addition array manual   automatic	0   0					
Automatic harvest valve	0					
Transfer group	0					
Speed controlled pumps	0					

Broad range of accessories available. Please contact us for further details.

• = included, -- = unavailable, 0 = option

# BIOSTAT<sup>®</sup> D-DCU – Technical Specification

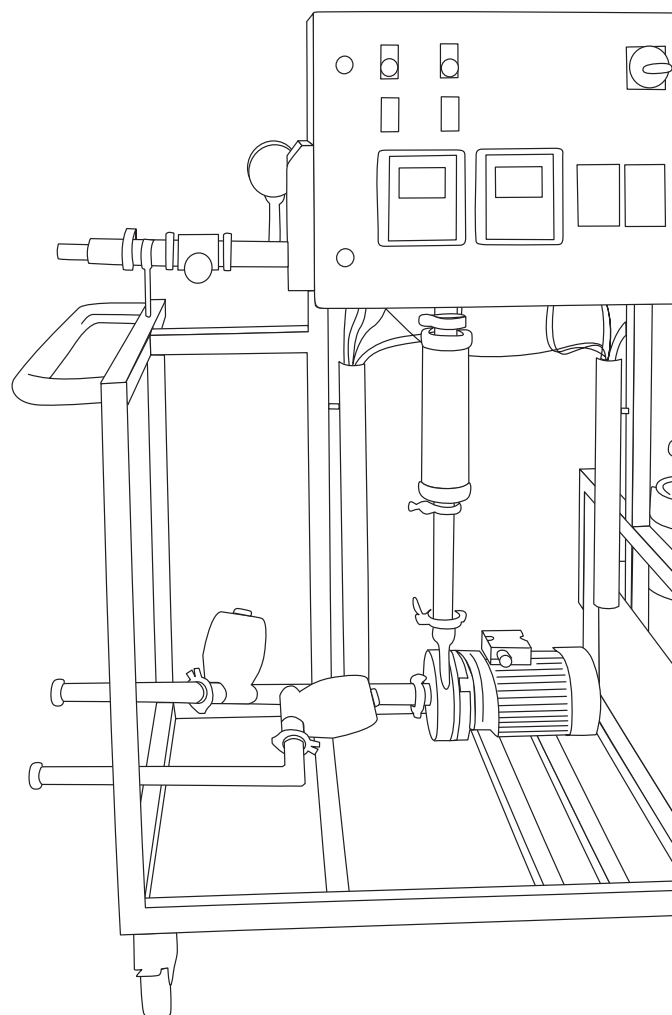
Technical Specification		10 L	20 L	30 L	50 L	100 L	200 L			
Space requirement Single [W × H × D]	["]	58.3 × 82.7 × 43.3	58.3 × 82.7 × 44.5	58.3 × 82.7 × 45.3	76.8 × 92.9 × 61.8	76.8 × 100.8 × 61.8	76.8 × 120.1 × 70.9			
	[m]	1.48 × 2.1 × 1.1	1.48 × 2.1 × 1.13	1.48 × 2.1 × 1.15	1.95 × 2.36 × 1.57	1.95 × 2.56 × 1.57	1.95 × 3.05 × 1.8			
Space requirement Twin [W × H × D]	["]	84.6 × 82.7 × 43.3	84.6 × 82.7 × 44.5	84.6 × 82.7 × 45.3	122 × 92.9 × 61.8	122 × 100.8 × 61.8	122 × 120.1 × 70.9			
	[m]	2.15 × 2.1 × 1.1	2.15 × 2.1 × 1.13	2.15 × 2.1 × 1.15	3.1 × 2.36 × 1.57	3.1 × 2.56 × 1.57	3.1 × 3.05 × 1.8			
Required wall opening dimensions [W × H]	["]	31.9 × 78.8	31.9 × 78.8	31.9 × 78.8	41.8 × 67	41.8 × 67	41.8 × 67			
	[m]	0.81 × 2	0.81 × 2	0.81 × 2	1.06 × 1.7	1.06 × 1.7	1.06 × 1.7			
Culture vessel weight (approx.)	[kg]	80	100	120	300	450	600			
Supply Unit weight (approx.)	[kg]	170	170	170	320	320	320			
Control Tower weight (approx.) Single   Twin	[kg]	160   205								
Ambient temperature   relative humidity (non-condensating)		5 – 40°C   85%								
Utilities Requirements		Conditions		Max. Flow	Vessel Size					
					10 L	20 L	30 L	50 L	100 L	200 L
Process Air MO   CC Sparger   Overlay		4 barg   58 psig, controlled, Class 2 (ISO 8573-1)		[L/min]	15   1/10	30   2/20	45   3/30	75   5/50	150   10/100	300   20/200
O <sub>2</sub> MO Sparger   CC Sparger   Overlay		4 barg   58 psig, controlled, prefiltered		[L/min]	15   1/5	30   2/10	45   3/15	75   5/25	150   10/50	300   20/100
CO <sub>2</sub> MO Sparger   CC Sparger   Overlay		4 barg   58 psig, controlled, prefiltered		[L/min]	N   A 1   5	N   A 2   10	N   A 3   15	N   A 5   25	N   A 10   50	N   A 20   100
N <sub>2</sub> MO Sparger   CC Sparger   Overlay		4 barg   58 psig, controlled, prefiltered		[L/min]	N   A 1   5	N   A 2   10	N   A 3   15	N   A 5   25	N   A 10   50	N   A 20   100
Utility steam		4 barg   58 psig, controlled, prefiltered		[kg/h]	15	15	15	50	90	160
Clean steam		1.5 barg   21.8 psig, controlled, prefiltered		[kg/h]	5	5	5	8	10	26
Cooling water		4 barg   58 psig, controlled (15°C) prefiltered		[L/min]	5	5	5	25	25	50
Cooling water return		Atmospheric pressure to 1.5 barg   21.8 psig		[L/min]	5	5	5	25	35	50
CIP, cleaning and rinsing fluid		1.5 barg   21.8 psig, controlled		[L/min]	on request			33	43	70
Condensate		Atmospheric pressure (max. Temp. 98°C)								
Instrument air		6 barg   87 psig, controlled								
Power supply (TNS net): 5 wire: 3 × phase, 1 × ground, 1 × neutral		208 VAC/24A (FI switch intern 300mA) or 400 VAC/20A (FI switch intern 300mA)								
Power supply for electrical heater (TNS net): 5 wire: 3 × phase, 1 × ground, 1 × neutral		208 VAC/16A or 400 VAC/10A								
Control Tower		Integrated DCU-Controller, Gassing System and Pumps Single or Twin configuration								
Controller		Industrial PC (Siemens)								
Housing material		Stainless steel AISI 304								
Display   Operation		Touch Panel 19"   Touch screen								
Host communication		Industrial Ethernet								
External connections		Expandable process I/O								
Balance connection		3 per vessel; expandable of up to 6 per vessel								
External Inputs   Off gas analyzer input		2 per vessel; Analog in (0 – 10 V)   2 per vessel Analog in (4 – 20 mA)								
External feed pumps		up to 4 per vessel; 2 per vessel; Analog out (0 – 10 V)								
Gassing System		Up to 6 integrated Mass Flow Controllers and Flowmeter								
MO application		Air aeration, O <sub>2</sub> -Enrichment or Gas Flow Ratio; Max. total flow rate: 1.5 vvm								
CC application		Advanced Additive Flow; Max. total flow rate: Overlay 1 vvm   Sparger 0.1 vvm								
Flow meter		Air calibrated @ 4 barg 20°C   scale lenght 120 mmm								
Flow range		0.12–1.06 L/min up to 70–330 L/min								
Accuracy		+/- 4% FS								
Thermal Mass Flow Controller		Air   N <sub>2</sub> , O <sub>2</sub> or CO <sub>2</sub> calibrated								
Flow range		0.02–1.0 sLpm up to 6–300 sLpm								
Accuracy		+/- 1% FS								
Integrated pumps		Up to 6 per vessel (2 × digital + 2 × digital   speed controlled + 2 × speed controlled)								
Pump head		Watson Marlow 114				Watson Marlow 314				
For tubings with 1.6 mm   1/16" wall thickness		For tubings with bore 0.5–4.8 [mm]   1/50–3/16["]				For tubings with bore 0.5–8.0 [mm]   1/50–5/16["]				
Rotation speed [rpm]		5	44	up to 200	6	60	up to 200			
Flow range	[mL/min] Bore	0.5 mm 1/50"	0.1	0.02 – 0.9	0.4 – 4	0.0 – 0.18	0.04 – 1.8	0.6 – 6		
		4.8 mm 3/16"	0.09 – 4.3	0.75 – 37.4	17 – 170	2.3 – 11.4	2.3 – 114	38 – 380		
		8.0 mm 5/16"	N   A	N   A	N   A	0.48 – 24	4.8 – 240	80 – 800		



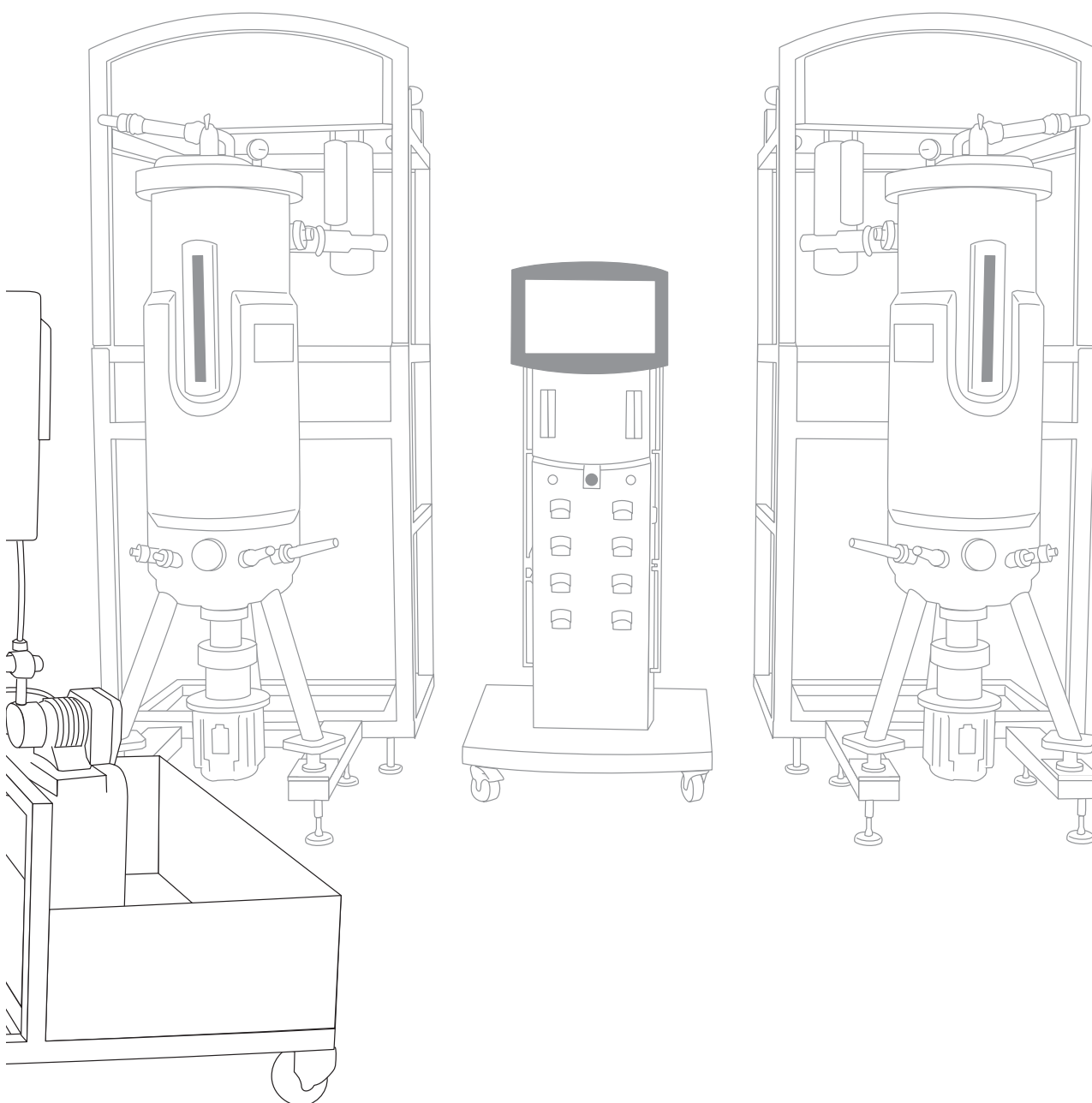
Supply Unit	Piping Skid in open frame design											
Material   Surface finish (product wetted parts)	Stainless steel AISI 316L   MO: Ra ≤ 0.8 µm (31.5 Ra or better)   CC: Ra ≤ 0.4 µm (15.7 Ra or better)											
Temperature control system – steam version	Closed loop thermostat system with recirculation pump, heat exchanger for cooling and heating or   and electrical heater											
Temperature control (operation   sterilization):	8°C above cooling water to 90°C   up to 130°C											
Heat exchanger (cooling   heating – steam version)	Stainless steel, copper soldered, optional stainless steel   welded version available upon request											
Electrical heater (optional)	6 kW (10–30 L: complete electrical heated; 50–200 L: auxiliary electrical heater only)											
Culture vessel	10 L		20 L		30 L		50 L		100 L		200 L	
H:D ratio	2:1	3:1	2:1	3:1	2:1	3:1	2:1	3:1	2:1	3:1	2:1	3:1
Total volume	14 L	15 L	29 L	31 L	42 L	41 L	74 L	77 L	152 L	152 L	313 L	323 L
Working volume	10 L	10 L	20 L	20 L	30 L	30 L	50 L	50 L	100 L	100 L	200 L	200 L
Minimal working volume	3.5 L	2.5 L	5.5 L	3.5 L	6.4 L	5.4 L	13 L	13L	24 L	24 L	47 L	41 L
Jacketed cylindrical part   Jacketed bottom	yes   no	yes   no	yes   no	yes   no	yes   no	yes   no	yes   no	yes   no	yes   yes	yes   no	yes   yes	yes   no
Weight lid with blind plugs [kg]	12	11	16	14	18	16	34	22	45	35	95	68
Agitation speed ranges for MO (max. impeller tip speed ≥ 5 m/s)	20– 1500	20– 1500	20– 1200	20– 1200	20– 1100	20– 1100	20– 900	20– 900	20– 700	20– 700	20– 570	20– 570
Motorpower   torque [kW   Nm]	2.3   5	2.3   5	3.1   9.4	3.1   9.4	3.1   9.4	3.1   9.4	4.2   16.2	4.2   16.2	4.9   26.7	4.9   26.7	6.6   48.2	6.6   48.2
Agitation speed ranges for CC (max. impeller tip speed ≥ 2 m/s)	350	N   A	300	N   A	260	N   A	220	N   A	180	N   A	130	N   A
Motorpower   torque [kW   Nm]	2.3   5	N   A	2.3   5	N   A	2.3   5	N   A	3.1   9.4	N   A	4.2   16.2	N   A	4.2   16.2	N   A
Impeller to vessel diameter [Rushton impeller]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Impeller to vessel diameter [3-blade segment impeller]	0.5	N   A	0.5	N   A	0.5	N   A	0.5	N   A	0.5	N   A	0.5	N   A
Lid ports	1 × sight glass for illumination 1 × port for Exhaust 9 × 19 mm port						1 × sight glass for illumination 1 × spare port DN 50 1 × port for CIP – connection 1 × port for Exhaust 8 × 19 mm port 3 × lifting eye					
Upper side wall	4 × 25 mm port 1 × Sparger aeration 1 × Overlay aeration   Bypass Sparger 1 × port for rupture disc   safety valve 1 × rectangular sight glass						3 × 25 mm port 1 × Sparger aeration 1 × Overlay aeration   Spare 1 × port for rupture disc 1 × spare DN50 1 × rectangular sight glass					
Lower side wall	5 × 25 mm port 1 × sanitary TC port 1 × port for temperature sensor						5 × 25 mm port 1 × sanitary TC port 1 × port for temperature sensor					
Bottom	1 × flange for agitator 1 × Harvest   Drain valve						1 × flange for agitator 1 × Harvest   Drain valve					
Jacket	1 × fluid in 1 × fluid out						1 × fluid in 1 × fluid out					
Vessel design	Jacketed stainless steel vessel with torospherical bottom and vertical sight glass Bottom agitation system											
Material (product wetted parts)	Stainless steel AISI 316 L   Borosilicat glass   EPDM (FDA approved)											
Surface finish product wetted	2:1 Vessel Ra ≤ 0.4 µm (15.7 Ra or better), electropolished   3:1 vessel: Ra ≤ 0.8 µm (31.5 Ra or better), electropolished											
Pressure design criteria vessel   jacket	–1/3 barg @ 150°C   –1/4 barg @ 150°C											
Sensors   measurement ranges   resolution												
Dissolved oxygen	Polarographic or optical   0–100%   1%   0.1%											
pH	Gel filled   2–12   0.01 pH											
Foam   Level   High Foam	Conductive probe, stainless steel ceramic isolated											
Temperature sensor Vessel/Jacket	Pt100   0–150°C   0.1 C / Pt100   0–150°C   0.1 C											
Redox	Gel filled   –1000 – 1000 mV   1 mV											
Pressure	Piezoresistive sensor   –0.5–2 [barg]   1 mbar											
Turbidity	Single Channel NIR Absorption Probe, 0–6 AU   0.01 AU											
Regulatory compliance	CE   UL   CSA (EN61010, UL61010); Culture vessel: ASME or PED or China pressure vessel regulation											

# NEW Mobile CIP Unit – Technical Specification

Technical Specification	
Design	Mobile stainless steel skid
Dimensions (approx.) [W × H × D]	72.9 × 64.6 × 30.7
	1851 × 1640 × 780
Weight (approx.)	100
Material   Surface finish (product wetted parts)	Stainless steel AISI 316L   Ra < 0.8 µm (31.5 Ra or better)   EPDM   PVC   PE   PTFE   others
Utility Requirements	Conditions   Flow
Water (WFI   PW   RO)	min. 1 barg
Drain	gravity   max. flow 3.5 m <sup>3</sup>
Drain CIP pump	gravity
Instrument air	6 barg   87 psig, controlled
CIP supply line	
CIP return line	
Power supply (TNS net): 5 wire: 3 + phase, 1 + ground, 1 + neutral	208 VAC   60 Hz or 400 VAC   50 Hz
CIP pump	Connection   Height
Type	TC 64   TC 2.5"   401 mm   15.8"
Material pump head	TC 64   TC 2.5"   684 mm   26.9"
Flow	TC 25   TC 1"   135 mm   5.31"
Valves	
Water	Quick coupling
Recirculation	TC 64   TC 2.5"
Drain	TC 64   TC 2.5"
Drain valve pump housing	



<b>Detergent dosing unit</b>	
Number of dosing units	1 (2nd optional)
Pump   Flow rate	Diaphragm pump   up to 25 L/h
Storage container volume   Material	40 L   PE
<b>Instrumentation   Measurement range</b>	
<b>Function</b>	
Conductivity sensor (high)	1010 – 2000 mS
Conductivity sensor (low)	0.04 – 500 µS
Level switch	Dry run protection for pump
Pressure gauge	– 1/5 bar
<b>External connections</b>	Connection to BIOSTAT® D-DCU for automated CIP sequence control (valves and recirculation pump)



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# HyPerforma Single-Use Mixer

## The next generation of efficiency and performance

The Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) provides you with enhanced functionality, ease of use, and efficiency. The complete HyPerforma S.U.M. system consists of a mixer tank with a motor controller, available in sizes of 50, 100, 200, 500, 1,000, and 2,000 L with a 5:1 turndown mixing ratio. The redesigned HyPerforma S.U.M. maintains traditional stirred-tank mixer design principles with a directly coupled motor impeller drive assembly and a cylindrical tank with a specific height-to-diameter ratio, allowing for quick turnaround times for liquid-to-liquid mixing and powder-to-liquid mixing.

## Critical upstream application steps

- Media preparation
- Final formulation steps
- Buffer preparation
- Harvest vessels
- Large-volume mixing (up to 2,000 L)

## Critical downstream application steps

- Pooling and liquid transfer
- Product suspension
- Mixing and storing multiple batches
- Buffer preparation
- Viral inactivation



## HyPerforma S.U.M. options

- Powder hanger for 1 kg, 5 kg, and 25 kg Thermo Scientific™ Powdertainer™ BioProcess Containers (BPCs)
- AC and DC motor options; electrical box only available on AC option
- 3x load cell weighing system with summing box (options for displaying signal output)
- Cable management system to organize process tubing lines
- Available in either water-jacketed or non-jacketed (no heat transfer) design
- pH and conductivity probes and display
- Open-top or closed-top Thermo Scientific™ BPC design
- BPCs available in Thermo Scientific™ CX5-14 Film and Aegis™5-14 Film options

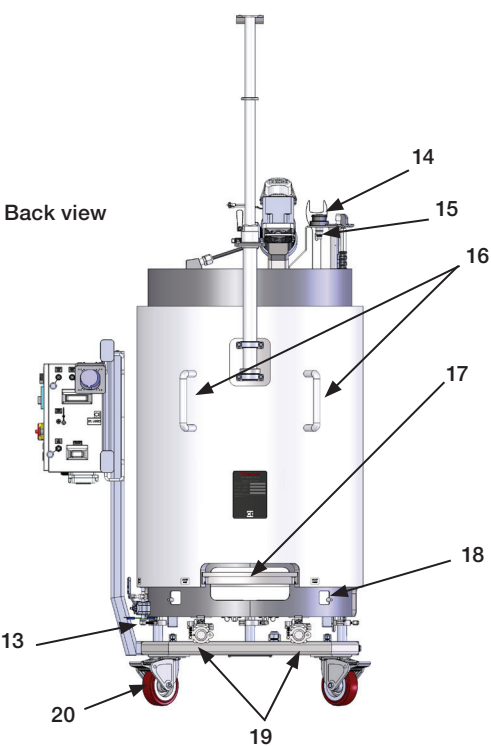
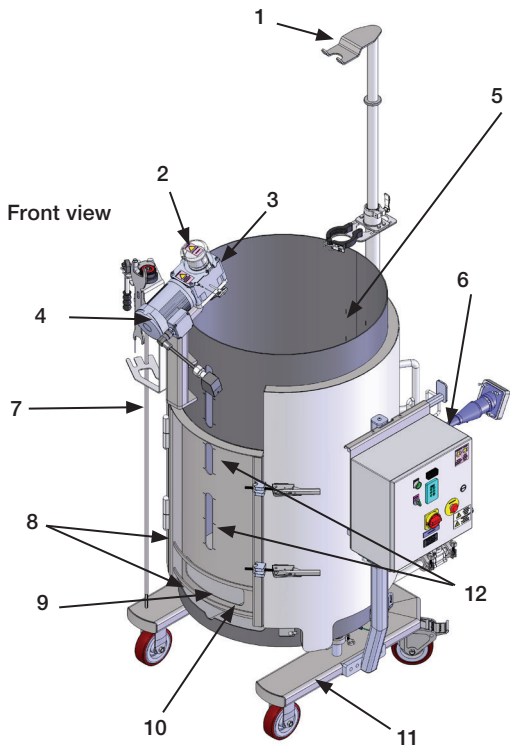
Options outside of the above list may be reviewed with a Thermo Fisher Scientific sales representative and considered for customizations to the standard mixer design.

**Standard S.U.M. hardware ordering information**

Note: Models without water jackets may have slightly different dimensions than the water-jacketed model shown here. See the drawings provided with your unit for exact dimensions for non-jacketed models. Non-jacketed models do not have the capability to heat or cool the liquid inside the tank.

Description	Cat. No.
Non-jacketed, DC motor, with load cell	SUM0500.9001
Jacketed, DC motor, with load cell	SUM0500.9002
Non-jacketed, 120 VAC, AC motor, with load cell	SUM0500.9003
Jacketed, 120 VAC, AC motor, with load cell	SUM0500.9004
Non-jacketed, 240 VAC, AC motor, with load cell	SUM0500.9005
Jacketed, 240 VAC, AC motor, with load cell	SUM0500.9006

**Design features**



1. Powder tainer arm (optional)

2. Mixing assembly with shield

3. Bearing port receiver with clamp

4. Mixer motor

5. Stainless steel (grade 304) outer support container

6. Electrical control panel (optional)

7. Driveshaft (stored)

8. 3/8 in. dimpled jacket (side and bottom)

9. Probe access window

10. Probe hanger bracket

11. Cart assembly
12. Liquid sight windows

13. Bleed valve (water-jacketed models only)

14. Standard tool set: 3/8-in., 150 in.-lb. square torque wrench, load cell and motor cap lockout wrench

15. Bearing hub (for open-top mixing only)

16. Handles

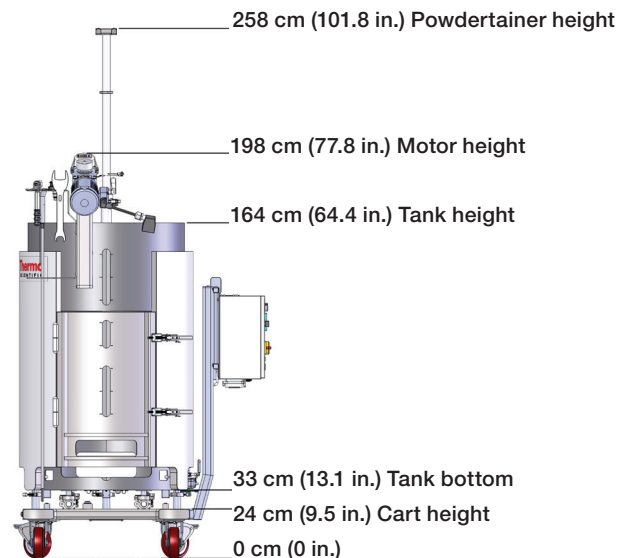
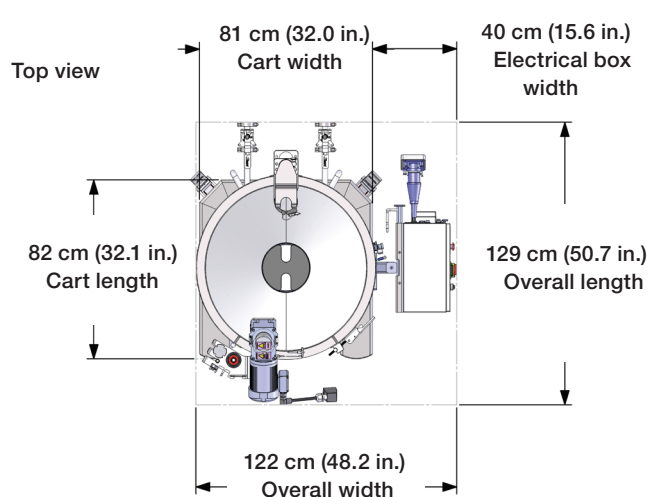
17. Cutout for BPC loading

18. Bottom cutouts/pins for BPC attachment and alignment

19. 1½ inch tri-clamp connection ports for water inlet/outlet (water-jacketed models only)

20. Casters (3 swiveling and 2 fixed)

Note: Models without water jackets include the same features as the water-jacketed models shown here, but without the jacket and inlet/outlet ports. Optional load cells and cable management system are not shown. See the accessories section for more information about these items.



## Standard 500 L S.U.M. hardware

### 500 L S.U.M. specifications

		AC motor		DC motor	
		Non-jacketed	Jacketed	Non-jacketed	Jacketed
Fluid jacket	Jacket area: full/half volume	–	2.2 m² (23.9 ft.²)/ 1.4 m² (14.8 ft.²)	–	2.2 m² (23.9 ft.²)/ 1.4 m² (14.8 ft.²)
	Jacket volume	–	11 L (2.9 gal)	–	11 L (2.9 gal)
	Jacket flow rate at 50 psi	–	96.8 L/min (25.5 gal/min)	–	96.8 L/min (25.5 gal/min)
	Process connection	–	1 in. sanitary tri-clamp	–	1 in. sanitary tri-clamp
	Temperature Control Unit (TCU) model: Maximum heating/cooling	–	TF2500: 2,800/2,500 W	–	TF2500: 2,800/2,500 W
	Approximate liquid heat-up time (5–37°C)	–	2.6 hr	–	2.6 hr
	Approximate liquid chill-down time (37–5°C)	–	3.7 hr	–	3.7 hr
Misc.	RTD or thermocouple, 1/8 in. (3.18 mm) OD	RTD: Pt-100 (standard)			
Support container	Overall width	122.5 cm (48.23 in.) with e-box		86.4 cm (34 in.) without e-box	
	Overall length	130.2 cm (51.25 in.) with e-box		130.2 cm (51.25 in.) without e-box	
	Overall height	251.1 cm (98.9 in.)			
	Dry skid weight (mass)	333 kg (734 lb)	431 kg (951 lb)	333 kg (734 lb)	431 kg (951 lb)
	Wet skid weight, rated working volume (mass)	833 kg (1,836 lb)	931 kg (2,053 lb)	833 kg (1,836 lb)	931 kg (2,053 lb)
General	Ceiling height required for standard driveshaft loading	320 cm (126 in.)			
	Ceiling height required for optional, non-standard 2-piece drive shaft loading	275.6 cm (108.5 in.)			
	Electrical power supply requirement (voltage, phase, current)	120/240 VAC, single, 20/10 A		Dependent on controller	
	pH and dissolved oxygen (DO) probe, autoclavable type (AppliSens™, Broadley James™, Mettler Toledo™)	12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread			
	Noise level	< 70 dB at 1.5 m			
Recommended operating parameters	Operating temperature range	2–40°C ±0.1°C (36–104°F ±0.2°F)			
	Motor speed	30–350 rpm			
	Volume range	100–500 L			
	Maximum BPC pressure	0.03 bar (0.5 psi)			
	Maximum BPC pressure during operation	0.007 bar (0.1 psi)			
	Continuous operating time	21 days mixing time at nominal volume only			



## Load cells

Load cells are typically radially mounted in sets of three. The mounting location varies slightly for each size in order to allow easy access to the bottom drain or sparging mechanisms and tubing.

## Load cell displays

Harsh mount load cell displays are available as an option for all HyPerforma S.U.M. systems. They are normally mounted on the electrical box or a wall and are available with four different interfaces.

## Powdertainer arm

A Powdertainer arm is available as an option for powder-liquid applications. It holds the container of powder above the mixer and attaches it to the BPC with a clamp. The arm adjusts vertically and swivels to enable convenient lifting of the Powdertainer onto the hanger.

## pH and conductivity monitoring devices

Mettler Toledo pH and conductivity sensors with a display monitor are available in single pH, dual pH, and pH and conductivity configurations. The monitor is mounted on the post behind the electrical box, and includes a color touch screen and control and alarm-management features.

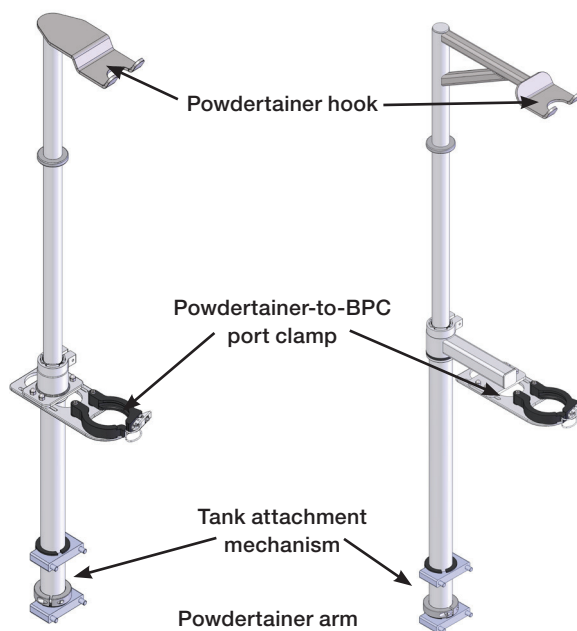
Description	Cat. No.
Cable management system (500 L)	SV50992.03
3x load cell with summation box, without display	SV50988.03
Mettler Toledo IND331 display, with analog interface (STD), 120 VAC U.S. line cord/plug	SV50177.306
Mettler Toledo IND331 display, with Allen-Bradley™ RIO interface, 120 VAC U.S. line cord/plug	SV50177.307
Mettler Toledo IND331 display, with DeviceNet interface, 120 VAC U.S. line cord/plug	SV50177.308
Mettler Toledo IND331 display, with Ethernet/IP and Modbus TCP interface, 120 VAC U.S. line cord/plug	SV50177.309
Mettler Toledo IND331 display, with Profibus interface, 120 VAC U.S. line cord/plug	SV50177.310
Powdertainer arm for 50–1,000 L mixers	SV51002.01
Mettler Toledo pH monitoring device, single channel	SV51004.01
Mettler Toledo pH monitoring device, dual channel	SV51004.02
Mettler Toledo pH and conductivity monitoring device, dual channel	SV51004.07



Mettler Toledo MTB load cell for 50–1,000 L S.U.M. units.



Mettler Toledo IND331 harsh-mount load cell display for all S.U.M. systems.



pH and conductivity monitoring device



Autoclave tray and probe assembly

The autoclave tray holds the electrochemical probes and bellows in place during the autoclave sterilization process. Design elements include the following:

- Fabricated from stainless steel
- Plastic handle provides for easy transport right out of the autoclave
- Positions probes on 15% incline for greater probe and membrane longevity
- Prevents probe bellows from collapsing during sterilization
- Probe holder accommodates two probes

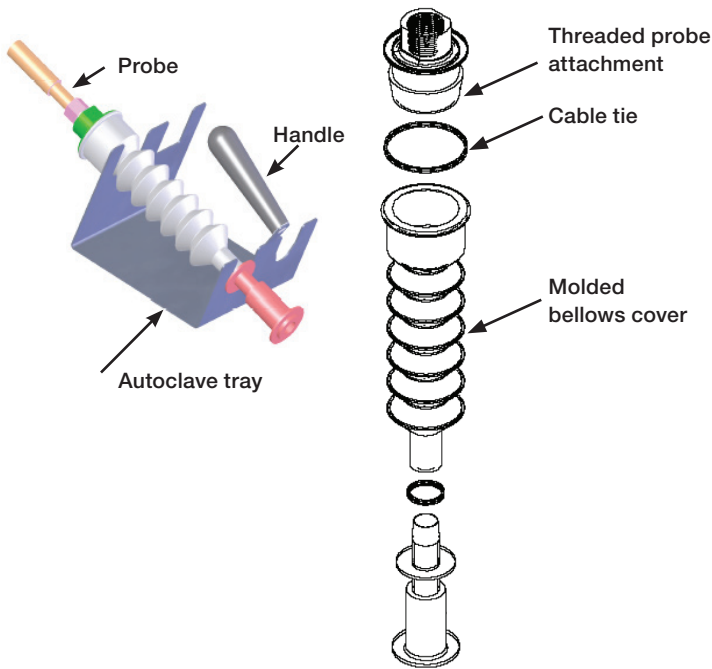
Probe clips

Stainless steel probe clips are used to hold the probes in place on the S.U.M. tank. The independently movable probe clips hang on a thin brace above the probe port tank cutout and are held in place by an adjustable spring plunger. The probes are inserted into the clip mechanism and held in place by a half-spring clip.

Heavy-duty tubing clamps

Heavy-duty clamps are used for pinching off line sets that are not in use in order to prevent process fluids from escaping. Prior to sterile probe insertion, tubing clamps must be in place to close off probe ports.

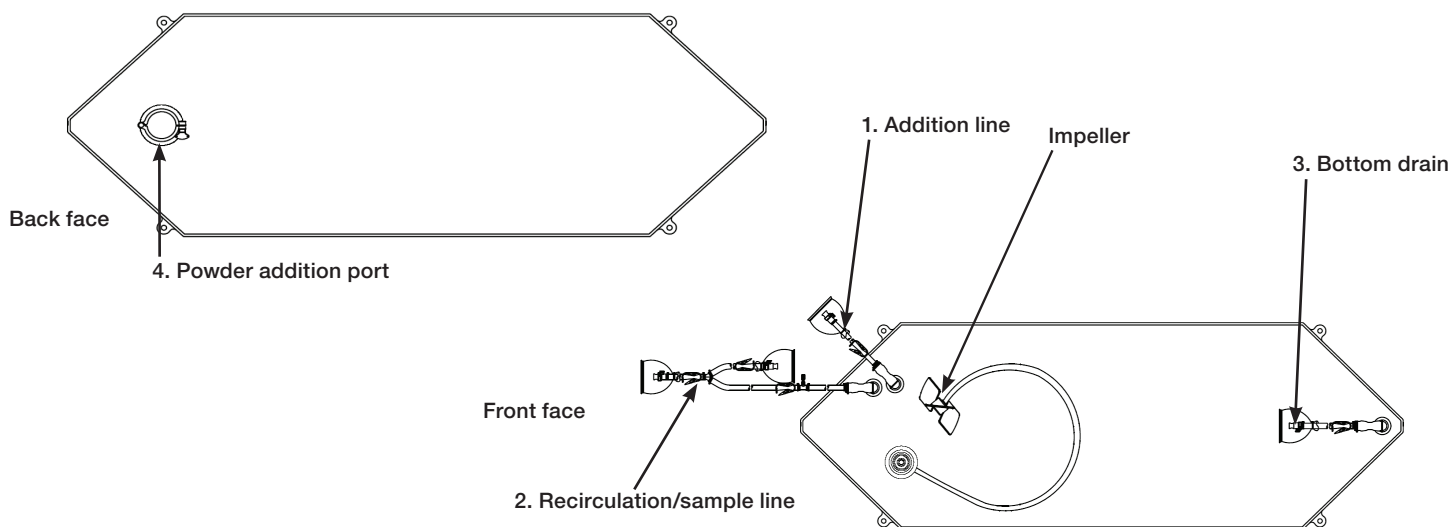
Description	Cat. No.
Autoclave tray (stainless steel with plastic carry handle)	SV50177.01
4 probe clips	SV50177.23
Heavy-duty tubing clamp (single)	SV20664.01
Heavy-duty tubing clamp (10 pack)	SV20664.04



Probe clip

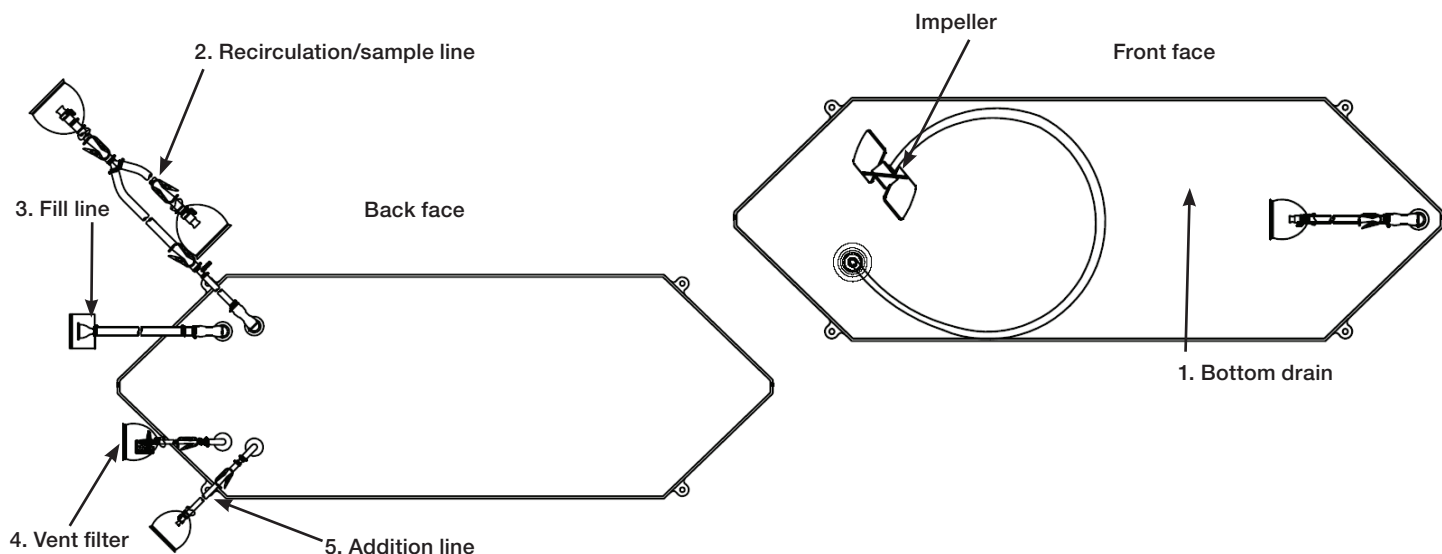


Heavy-duty tubing clamps



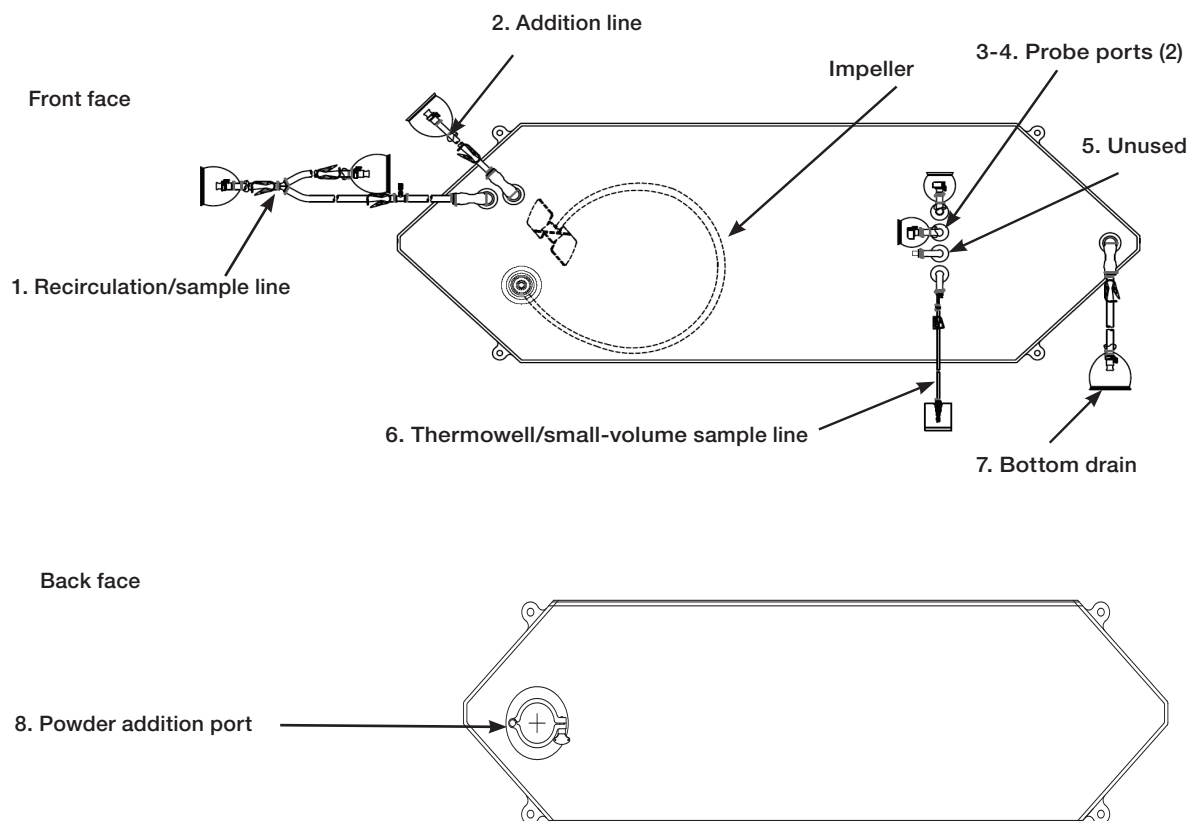
### Standard 500 L BPC for powder–liquid applications without probe ports

Line	Description	Tubing set (inner diameter x outer diameter x length)	End treatment
1	Addition line	12.7 mm (1/2 in.) x 15.9 mm (5/8 in.) C-Flex™ x 152 cm (60 in.)	Plugged 12.7 mm (1/2 in.) MPX body
2	Recirculation/sample line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 137 cm (54 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.)	Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body
3	Bottom drain	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.)	Capped 12.7 mm (1/2 in.) MPX body
4	Powder addition port	7.6 mm (3 in.) sanitary fitting, tri-clamp tube ports	Capped with gasket



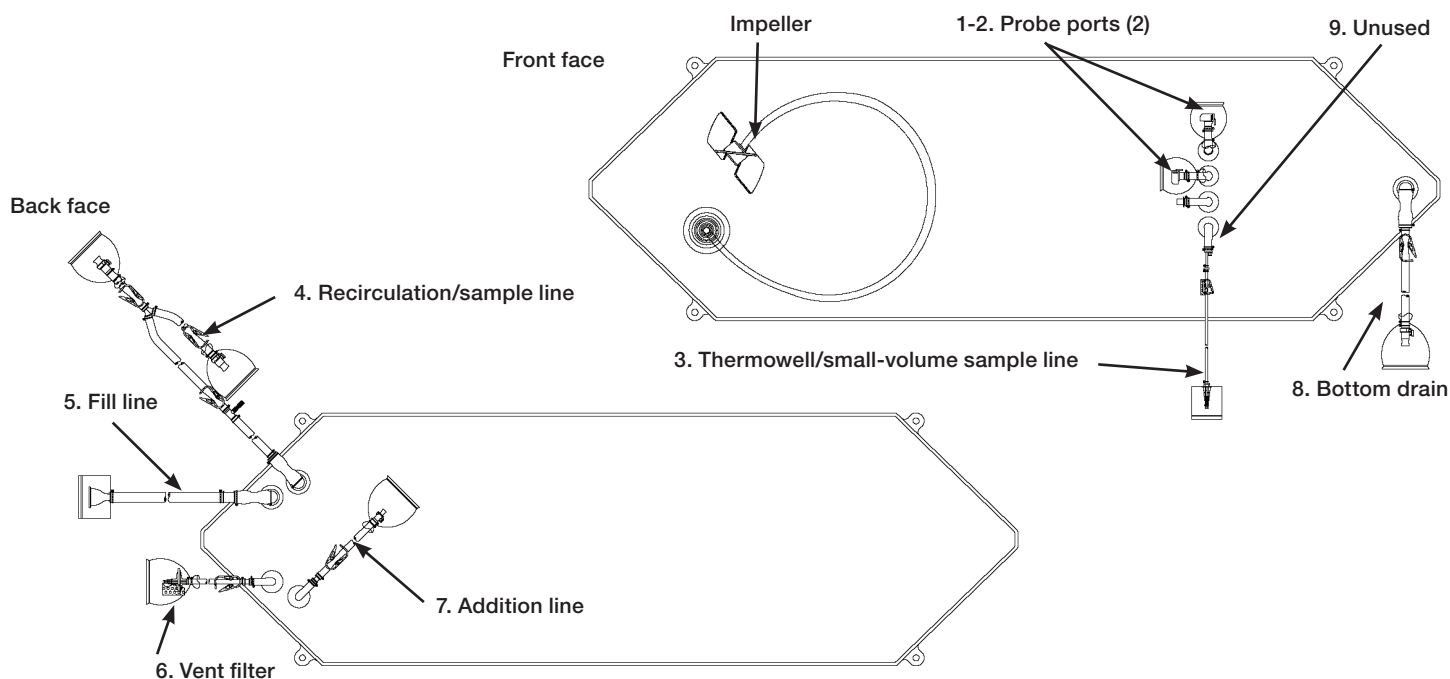
### Standard 500 L BPC for liquid–liquid applications without probe ports

Line	Description	Tubing set (inner diameter x outer diameter x length)	End treatment
1	Bottom drain	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.)	Capped 12.7 mm (1/2 in.) MPX body
2	Recirculation/ sample line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 168 cm (66 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.)	Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body
3	Fill line	19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex x 152 cm (60 in.)	38.1 mm (1 1/2 in.) tri-clamp (SterilEnz™)
4	Vent filter	6.4 mm (1/4 in.) x 12.7 mm (1/2 in.) C-Flex x 10.2 cm (4 in.)	Sterile hydrophobic vent filter (0.2 µm PVDF, Acro™ 50)
5	Addition line	9.5 mm (3/8 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.)	Plugged 9.5 mm (3/8 in.) MPX insert



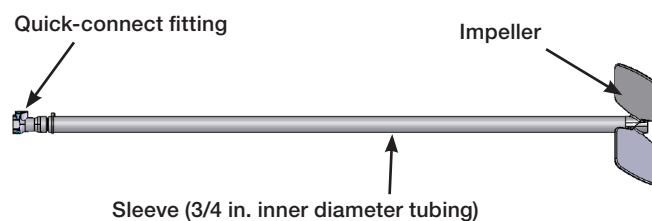
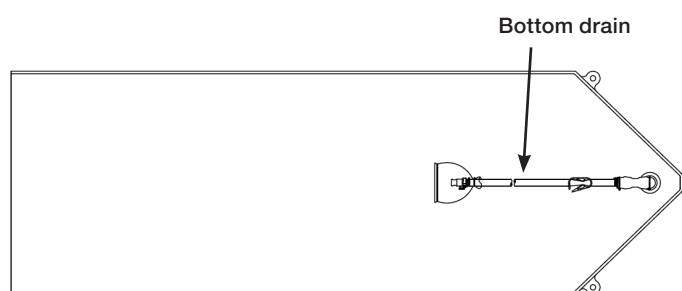
### Standard 500 L BPC for powder-liquid applications with probe ports

Line	Description	Tubing set (inner diameter x outer diameter x length)	End treatment
1	Recirculation line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 137 cm (54 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.)	Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body
2	Addition line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.)	Plugged 12.7 mm (1/2 in.) MPX insert
3-4	Probe ports (2)	None	Kleenpak™ aseptic connector KPCHT series (female)
5	Unused	None	Plug
6	Thermowell/ small-volume sample line	Thermowell adapter for 3.2 mm (1/8 in.) diameter 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) x 30 cm (12 in.)	Luer and SmartSite™ valve port
7	Bottom drain	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.)	Capped 12.7 mm (1/2 in.) MPX body
8	Powder addition port	7.6 mm (3 in.) sanitary fitting, tri-clamp	Cap with gasket



### Standard 500 L BPC for liquid-liquid applications with probe ports

Line	Description	Tubing set (inner diameter x outer diameter x length)	End treatment
1—2	Probe ports (2)	None	Kleenpak aseptic connector KPCHT series (female)
3	Thermowell/ small-volume sample line	Thermowell adapter for 3.2 mm (1/8 in.) diameter 3.2 mm (1/8 in.) x 6.4 cm (1/4 in.) C-Flex x 30 cm (1/2 in.)	Luer and SmartSite valve port
4	Recirculation line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 168 cm (66 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 30 cm (12 in.)	Capped 12.7 mm (1/2 in.) MPX insert Plugged 12.7 mm (1/2 in.) MPX body
5	Fill line	12.7 mm (1/2 in.) x 25.4 mm (1 in.) C-Flex x 152 cm (60 in.)	38.1mm (1 1/2 in.) tri-clamp (SterilEnz)
6	Vent filter	6.4 mm (1/4 in.) x 12.7 mm (1/2 in.) C-Flex x 10.2 cm (4 in.)	Sterile hydrophobic vent filter (0.2 µm PVDF, Acro 50)
7	Addition line	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex x 61 cm (24 in.)	Plugged 12.7 mm (1/2 in.) MPX insert
8	Bottom drain	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex x 122 cm (48 in.)	Plugged 12.7 mm (1/2 in.) MPX body
9	Unused	None	Plug



### Standard open-top liners

Description	Cat. No.
500 L standard open-top liner, 0 probes, CX3-9 film	SH30762.06

### Standard impeller sleeve

Description	Cat. No.
500 L impeller sleeve for open-top mixing*	SH30749.10

\* The bearing hub needed for open-top mixing is automatically supplied with the tank hardware.

## Custom BPC products

Category	Options/capability	Notes
Tubing type	C-Flex, platinum-cured silicone, PVC, PharMed™, PharmaPure™.	More information is available in the tubing selection guide.
Tubing size	Ranges from 3.18 mm (1/8 in.) to 25.4 mm (1 in.) inner diameter in various lengths.	More information is available in the tubing selection guide.
Connectors	Luer, Colder Products Company™ (CPC) quick connects, SIP connectors, tri-clamp, Kleenpak, SmartSite, Clave™, Lynx™ steam-thru, CPC steam-thru, Gore™ steam valve, Gore™ Mini TC, BioQuate™, SterilEnz, end plug.	More information is available in the connection system selection guide. Note: the only option for probe port connections is Kleenpak connectors.
Probe ports/line addition ports	Ports may be added if they are compatible with the hardware.	The reusable probe port connection uses a Kleenpak connector.
Disposable sensors	Pressure sensor: PendoTECH™ and Finesse Solutions (PendoTECH comes standard on 500 L and 1,000 L S.U.M.); DO and pH sensor: Finesse Solutions and PreSens™; pH sensor: Mettler Toledo.	Choice of qualified vendors available.
Port sizes	Limited engineer-to-order customization only.	Dependent on location in BPC and fit with hardware (e.g., 1 in. ID port on harvest line).
Rearrangement of lines on existing ports	Limited customization possible, such as moving sample/thermowell port to a probe tube port, or swapping exhaust outlet line with liquid lines.	Dependent on location in BPC and fit with hardware.
Dip tube lines	Limited customization possible.	Length cannot interfere with impeller and shaft.
Filters on media and supplement inlets	Limited engineer-to-order customization only. Choice of filters used to sterilize incoming media or supplements are available.	

Note: Not all options are available for all ports. It is not possible to customize port type, port location, chamber dimensions, or mixing assembly. For additional information, please see the selection guides in the BPC Catalog.

## BPC packaging

Description	Details
Outer packaging	Supplied “flat-packed” with two polyethylene outer layers
Label	Description, product code, lot number, and expiry date on outer packaging and shipping container
Sterilization	Irradiation (25 to 38 kGy) inside outer packaging
Shipping container	Durable cardboard carton
Documentation	Certificate of analysis provided with each lot for each delivery

## Ordering information

500 L S.U.M. BPC	Size	Probe ports*	Film type	Cat. No.
Standard powder-liquid BPC	500 L	0	CX5-14	SH30751.01
	500 L	0	Aegis5-14	SH30973.03
Standard liquid-liquid BPC	500 L	0	CX5-14	SH30754.01
	500 L	0	Aegis5-14	SH30983.03
Standard powder-liquid BPC*	500 L	3	CX5-14	SH30751.02
	500 L	3	Aegis5-14	SH30974.03
Standard liquid-liquid BPC*	500 L	3	CX5-14	SH30754.02
	500 L	3	Aegis5-14	SH30982.03

\* All 500 L BPCs with probe ports are designed to allow probes to work properly at 5:1 turndown levels. These BPCs are only compatible with the HyPerforma hardware shown in this document. If you are using an older version of the S.U.M. hardware, do not use these items; instead refer to the legacy S.U.M. user manual or data sheets.

Find out more at [thermofisher.com/sum](https://thermofisher.com/sum)

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# Culturefuge 100

## Solids ejecting centrifuge

Many new biological products are derived from fragile organisms. Although relatively easy to separate the trick is accomplishing the separation in a gentle manner without destroying the shear sensitive cell wall membranes that isolate the complex intracellular proteins from the extracellular liquid. If this can be avoided, downstream purification of the target proteins becomes much easier.

### Applications

The machine is designed for clarification duty. Especially when clarifying liquids from shear sensitive particles. Applications that requires low oxygen pick-up can also take advantage of the hermetic features this machine offers.

### Standard design

The machine consists of a frame that has a horizontal drive shaft, worm gear, lubricating oil bath and hollow vertical bowl spindle in the lower part. The bowl is mounted on top of the spindle, inside the space formed by the upper part of the frame, the ring solids cover, the collecting cover, and the frame hood. The liquid discharge system also rests on this structure. All parts in contact with the process liquid are made of stainless steel. The bowl is of the solids-ejecting disc type with an automatic hydraulic operating system for discharging. It is a so-called timer triggered partial discharge system, meaning that only part of the bowl content is emptied during pre-set discharge intervals. The discharge takes place at full speed without any interruption of the feed. The centrifuge is available with main connections as sanitary flanges and all other utility connections clamp type. The electric motor is of standard type and has a built-in variable frequency drive. The design conforms with a number of EC directives, and machine is made in accordance with the general directives for machinery. Finally, the centrifuge is equipped with nozzles for flushing of the bowl top, the bowl bottom and the cyclone.

### Standard equipment

Each Culturefuge 100 centrifuge comes with control unit,



Fig. 1 Culturefuge 100 centrifuge

electric motor, in- and outlet connections, spare parts kit and set of tools.

### Material data

Bowl body, hood and lock ring	s.s. 1.4462 UNS S31803
Solids cover and frame hood	s.s. ASME SA-240 UNS 31603
Cyclone	s.s. ASME SA-240 UNS 31603
Bottom frame	Cast grey iron
In and outlet	s.s. mostly 1.4401 UNS 31600
Gaskets and O-rings	EPDM rubber (FDA approved)

## Operating principles

The feed is introduced to the rotating centrifuge bowl (fig 2) from the bottom through a hollow spindle (1), and is accelerated in a distributor (2) before entering the disc stack (3), where the separation takes place. The separated liquid phase leaves through the liquid outlet (4) at the top of the bowl. The collected solids in the solid space (5) are intermittently discharged from the periphery of the bowl. During normal production the operating water keeps the sliding bowl bottom (6) closed against the bowl hood (7). During discharge the sliding bowl bottom drops for a short time (less than a second) and the solids are ejected through the discharge ports (8). The high velocity of the ejected solids is reduced in the cyclone.

## Available models

The Culturefuge 100 centrifuge is available in pressure vessel designs according to ASME or to PED. In addition, different surface finish executions are available:

Bowl spindle	Ra 0.8
Bowl spindle	Ra 0.5 and electropolished
Machine top part	Inside: Ra 0.8, Outlet cover: Ra 0.5 and electropolished
Machine top part	Inside: Ra 0.8, Outlet cover: Ra 0.8
Machine top part	Inside: Ra 1.2, Outlet cover: Ra 1.2
Separator bowl	Inside: Ra 0.5 and electropolished, Outside: Ra 0.8
Separator bowl	Inside: Ra 0.8, Outside: Ra 0.8
Separator bowl	Inside: Ra 1.2, Outside: Ra 1.2

## Dimensions (approximate)

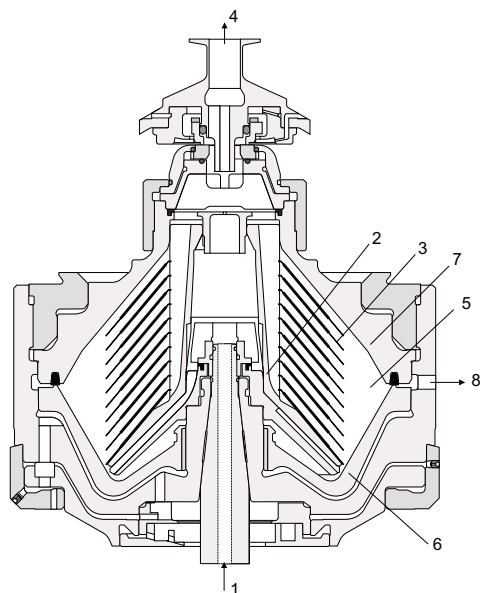
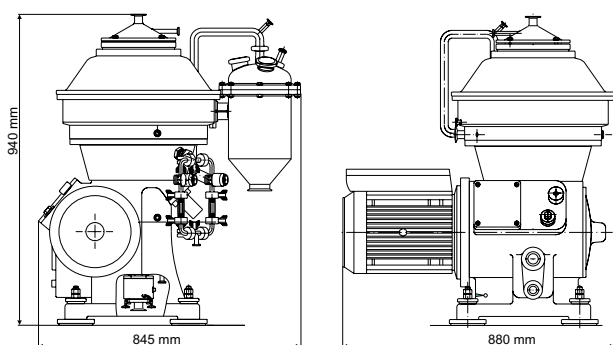


Fig. 2 Typical bowl for a hermetic solids-ejecting centrifuge. The details illustrated do not necessarily correspond to the centrifuge

## Technical specification

Hydraulic capacity	max. 2.0 m <sup>3</sup> /h <sup>1)</sup>
G-force	max. 12200 g
Bowl speed	max. 9650 rpm
Motor power installed	7.5 kW
Sound pressure	74 dB(A) <sup>2)</sup>
Overhead hoist lifting capacity	min. 100 kg

<sup>1)</sup> Actual capacity depends on feed material and separation demands

<sup>2)</sup> In compliance to EN ISO 4871

## Utilities consumption

Electric power	5.5 kW
Operating water	0.3 l/discharge
Cyclone flush	0 - 8 l/discharge
Cooling for seals	max. 300 l/h
Flushing above the bowl	0 - 1 l/discharge
Flushing under the bowl	0 - 1 l/discharge
Steam per sterilization cycle	5 - 10 kg

## Shipping data (approximate)

Centrifuge with bowl with motor	450 kg
Gross weight	600 kg
Volume	1.0 m <sup>3</sup>

## How to contact Alfa Laval

Contact details for all countries are continually updated on our website. Please visit [www.alfalaval.com](http://www.alfalaval.com) to access the information direct.





Life Sciences

USD 2719a

## Resolute® Manual Chromatography Columns



### *Process columns for optimized, contained, and scalable operation*

- ▶ High resolution flow path provides optimum efficiency, capacity, and peak symmetry.
- ▶ Central nozzle valve provides all column functions required for packing, unpacking, and running the column within a closed system.
- ▶ Scalable, reproducible packing methods reduce validation efforts as processes scale up.
- ▶ Common design from 280 to 1200 mm diameter (and up to 2000 mm on request).
- ▶ Designed to minimize packing events and buffer usage to optimize process economics.
- ▶ Options for manual or remote controlled nozzle valves.

#### **Applications**

Resolute columns have proven performance with a wide range of chromatography sorbents and chromatographic modes including ion exchange, mixed-mode, affinity and hydroxyapatite.

*Filtration. Separation. Solution.<sup>SM</sup>*

## Principle of Slurry Transfer

Resolute columns can be packed and unpacked with the column fully assembled. At the center of the operation is a nozzle valve in both the top and bottom of the column. Process liquids can enter and exit the column via either nozzle valve depending on which packing method is employed. Top and bottom nozzle valves are identical, making the flow profile the same in either direction. The ability to pack media with the top end cell in place contributes to process hygiene by reducing exposure to the external environment.

There are three positions for each nozzle valve (Figure 1).

### Effective Column Packing

#### Contained Operation

All column operations are performed in a closed system; therefore, there is less risk of the operator coming into contact with hazardous materials or the process being exposed to contamination. This results in improved safety and hygienic operation of the column process.

#### Reproducibility

By eliminating labor intensive column handling operations, the packing parameters can be set and applied within a standard operating procedure. This improves the reproducibility of the packed column performance.

#### Scalability

All design elements within the column mobile phase flow path are maintained throughout the range (up to 2000 mm diameter). Unlike conventional columns that are based on different seal and distributor cell designs as size increases, the Resolute column sealing arrangement and flow path follow a single design principle that delivers proven and consistent column performance.

#### Reduced Process Validation

Resolute columns and packing systems maintain identical linear velocities and pressure profiles during the packing process, whether in a 400 mm pilot-scale column or a 2000 mm process-scale column. Using the same packing methods reduces the effort required during preparation of standard operating procedure (SOP) for large-scale manufacturing packing methods.

**Figure 1**  
*Nozzle valve in pack position*



*Nozzle valve in run position*



*Nozzle valve in unpack position*



## Optimized for Efficiency and Scale Up

Resolute columns are equipped with Pall's proprietary high resolution mobile phase flow path which consists of a mobile phase flow path within the nozzle valve body, distribution end cells, and a fully flushed piston seal arrangement. The performance of these important column components is essential for optimal process efficiency, capacity, and peak symmetry.

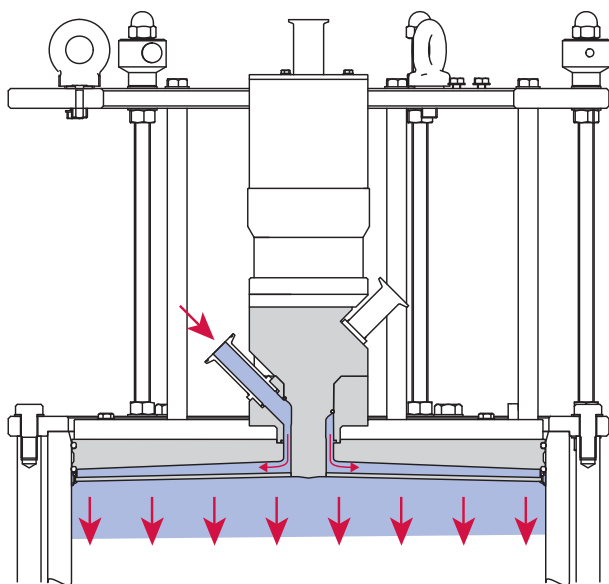
Resolute columns maintain identical design parameters throughout both the standard column range (from 280 mm to 1200 mm diameter) and the larger capacity columns (up to 2000 mm diameter). Operation at high linear velocities is free from non-linear column effects and assures true linear scalability of column performance, enabling users to scale up simply without method redevelopment or impact on the reproducibility of the packing process.

Key flow path features:

- ▶ Nozzle valve mobile phase path delivers consistent low pressure drop across column for true linear scale up.
- ▶ Distribution with coned end cell channels provides near plug flow conditions.
- ▶ Secondary cone on support ribs provides unimpeded route via top central nozzle for simple and effective removal of entrapped air as well as effective recovery of slurry via bottom central nozzle when unpacking.
- ▶ Nozzle tip retracts flush with bed support with no protrusion into packed bed.

**Figure 2**

*High resolution mobile phase flow path*



## Consistent Chromatography Performance

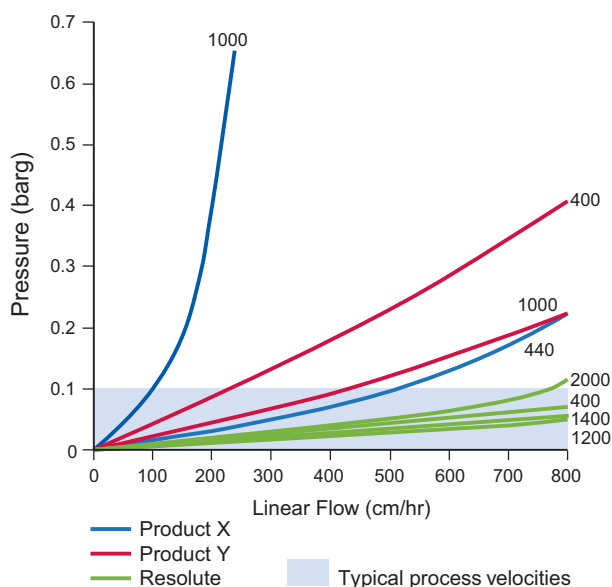
Resolute columns' high resolution mobile phase flow path enables operation at high linear velocities up to 1000 cm/h. The flow distribution cells and mobile phase flow path support near plug flow conditions irrespective of column diameter. Figure 3 shows pressure flow curves for Resolute columns and alternative manufacturers' columns. The columns were filled with water, and pressure and flow instruments were used to measure pressure drop across the filled column under process flow conditions. Resolute columns exhibit identical pressure flow curves, linear up to 1000 cm/h, at which the pressure drop is 0.1 barg (1.5 psig). Alternative column designs exhibit high, exponential pressure flow curves. During normal operations, actual pressure drop will be the sum of the value shown below and the additional value from the packed bed.

Resolute columns support true linear scale up of both the purification performance and column packing methods:

- ▶ Elimination of non-linear interference from column design enables monitoring of actual column pressure resulting from media bed properties.
- ▶ Pressure versus bed height relationship for a specific media, packed at the same compression, remains constant up to at least 1000 cm/h linear flow in any diameter of Resolute column.

**Figure 3**

*Pressure vs. flow for Resolute columns and alternative columns fitted with stainless steel mesh bed supports (column Ø mm)*





## Designed for Durability, Reliability, Versatility and Ease of Use

Resolute columns are available in diameters from 280 mm to 1200 mm, with 200 mm bed height adjustment and choice of bed heights up to 600 mm. Alternative bed height adjustment and diameters up to 2000 mm are available on request. Resolute manual columns feature:

- ▶ Manually operated nozzle valves. (See column options for pneumatically-actuated nozzle valves.)
- ▶ Choice of bed supports in polyethylene or 316L stainless steel.
- ▶ Acrylic column tube provides visibility of the packed bed.
- ▶ Simple, constant compression, adjuster seal, and precision bore eliminate need for additional mechanical or pneumatic activation of adjuster seals.
- ▶ Stainless steel support frame equipped with lockable castors (available up to 1000 mm column).

**Figure 4**

*Resolute manual 1000 mm internal diameter (ID) column*



## Sanitary Design

Sanitary design features ensure the longevity, consistency, and reliability of your process:

- ▶ Fully flushed flow path and adjuster seal facilitate clean-in-place (CIP).
- ▶ Minimum dead space fixed cell seal arrangement.
- ▶ Non-metallic mobile phase flow path is ideal for high salt, low pH conditions, and eliminates the risk of corrosion within the nozzle valve.
- ▶ Phthalate-free acrylic tube and peroxide-cured EPDM (ethylene-propylene-diene monomer) seals.
- ▶ Low-profile central nozzle retaining ring on the bottom bed support reduces media hold-up during unpacking.

**Figure 5**

*Manual nozzle*

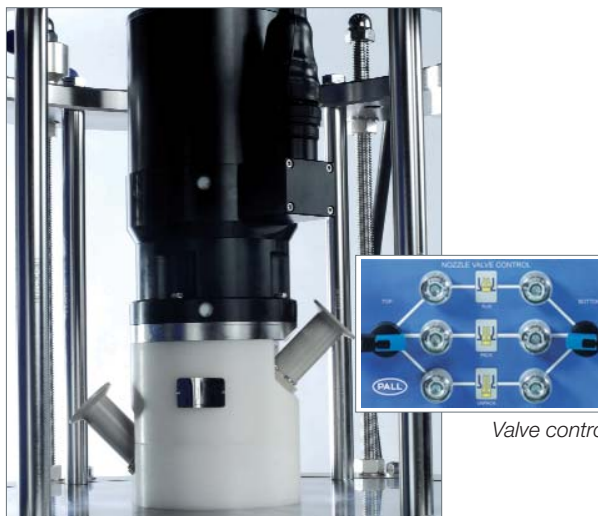


## Column Options

- ▶ Remote controlled pneumatic nozzle valve: a pneumatic actuator positions the nozzle to pre-set positions (Run, Pack, Unpack) with pneumatic controller and position feedback for increased process security. (Not available on 280 mm column.)
- ▶ Bed supports are available in stainless steel (10/20/50  $\mu$ m) and polyethylene (10/20/60  $\mu$ m), which offers a lower-cost alternative when frequent bed support changes are required.
- ▶ Robust stainless steel column tube with increased chemical compatibility. (Not available on 280 mm column.)
- ▶ Fixed bed capacity with no bed height adjustment available for media-specific or process requirements.
- ▶ Extended adjuster range (300 mm) adds packing flexibility.

**Figure 6**

*Remote controlled pneumatic nozzle*



*Valve control*

*Valve actuator*

## Column Specifications

Maximum allowable pressure	280 mm Ø column: 5.0 barg (72.5 psig); 400–1200 mm Ø column: 4.2 barg (60.9 psig)
Operating temperature	2–30 °C
Bed support type and rating	Stainless steel mesh: 10, 20, 50 µm; Polyethylene sinter: 10, 20, 60 µm
Stainless steel surface finish	Product flow path: < 0.6 µm Ra, electropolished Exterior components: < 0.9 µm Ra, electropolished Pressure retaining plates: < 1.5 µm Ra, 240 (UK) grit sateen Column frame: bright polished
Adjuster seal	Precision bore with compression seals
Media transfer nozzle	Manual or pneumatic remote controlled

## Materials of Construction

Process Wetted Components	Material
Column tube	Acrylic
Distribution cell	Polypropylene
Nozzle body	280–1000 mm Ø column: polyvinylidene fluoride (PVDF); 1200 mm Ø column: polypropylene
Mobile phase termination	280–1000 mm Ø column: polyetheretherketone (PEEK); ≥1200 mm Ø column: stainless steel 316L
Slurry nozzle tip	PEEK
Slurry inlet port	280 mm Ø column: PEEK; 400–1200 mm Ø column: stainless steel 316L
Bed support	Polyethylene sinter or stainless steel mesh
Main seals	EPDM (peroxide cured)
Nozzle tip seals	Fluorinated ethylene propylene (FEP) encapsulated silicone
Wiper ring	Polytetrafluoroethylene (PTFE)
External Components	
Stand	Stainless steel 316L
Castor	Stainless steel 304L
Wheel	280–600 mm Ø column: polyurethane; 800–1000 mm Ø column: nylon
Foot	1200 mm Ø column: stainless steel 316L

## Design

The basis of design for Resolute columns is PD5500. Columns, where applicable, are CE marked in accordance with the European Pressure Equipment Directive 97/23/EC.

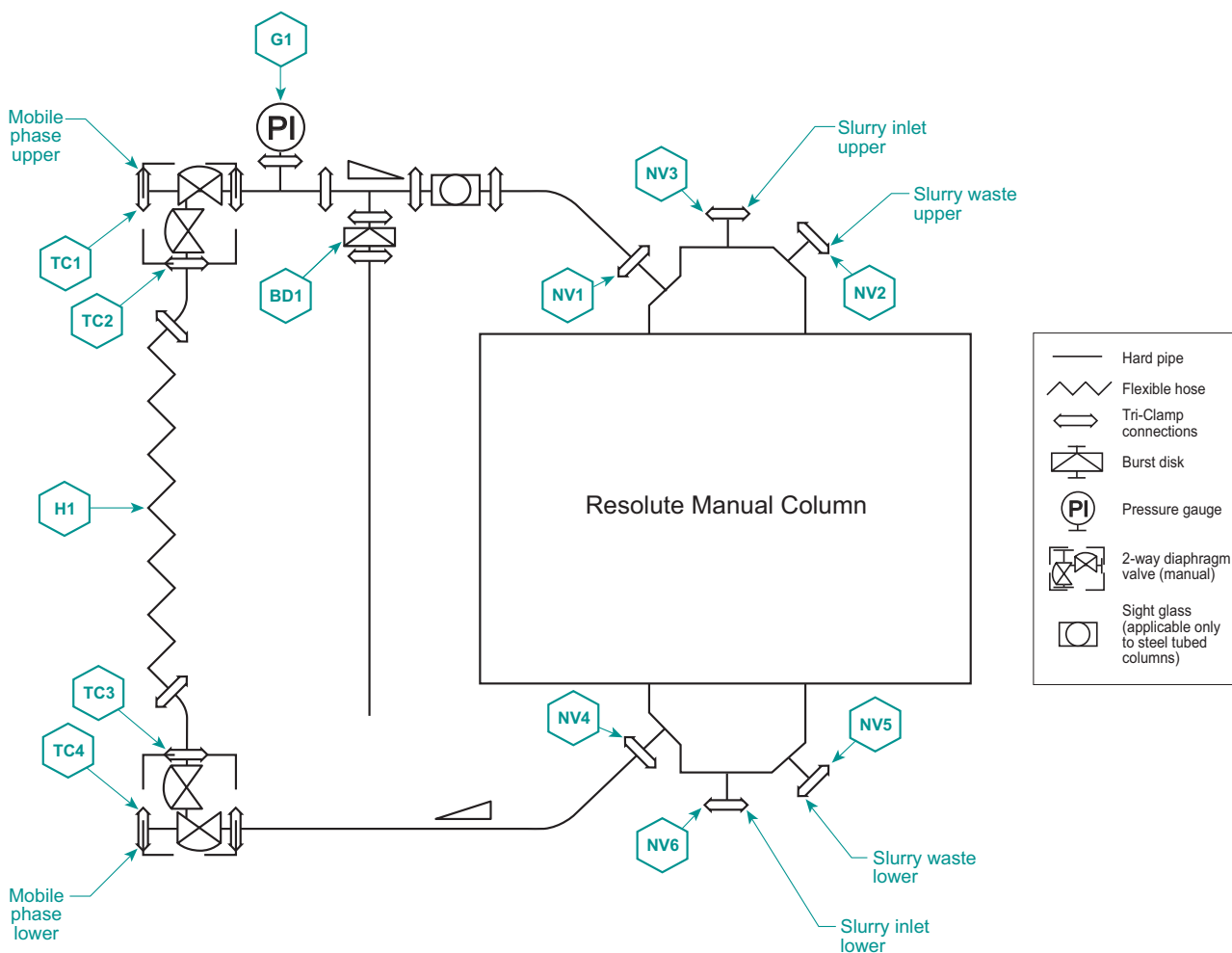
## Port Sizes and Pressure Flow Kit (PFK)

Below are the termination sizes for the column and PFK, and a schematic showing the scope of the column PFK kit. All connections are sanitary Tri-Clamp♦ connections with EPDM gaskets. PFK kits are available to complement the standard columns and are also available within an 8 weeks lead time.

Note that the standard PFK does not include sight glasses.

**Figure 7**

Scope of column PFK kit and termination sizes for column and PFK



Column Ø (mm)	Nozzle Valve Termination Size*						Pressure Gauge	Burst Disk	Block Valve Termination Size*				Flexible Hose Size*
	NV1	NV2	NV3	NV4	NV5	NV6	G1	BD1	GEMU♦ 3-Way Manual Diaphragm Valve 673 / 671 Bonnet				Pharmaline♦
									TC1	TC2	TC3	TC4	H1
300	0.5 in.	0.5 in.	0.5 in.	0.5 in.	0.5 in.	0.5 in.	1.0 in.	1.0 in.	0.5 in.	0.5 in.	0.5 in.	0.5 in.	0.5 in.
400-450	0.75 in.	0.75 in.	0.75 in.	0.75 in.	0.75 in.	0.75 in.	1.0 in.	1.0 in.	0.75 in.	0.75 in.	0.75 in.	0.75 in.	0.75 in.
600-630	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.	1.0 in.
800-1000	1.5 in.	1.5 in.	1.5 in.	1.5 in.	1.5 in.	1.5 in.	1.0 in.	1.5 in.	1.5 in.	1.5 in.	1.5 in.	1.5 in.	1.5 in.

\* (ASME BPE standard)

## Chemical Compatibility

Substance/Concentration	Acrylic	SS	PVDF	PP	EPDM/FEP	PE	PEEK	PTFE
Acetic acid < 1 M	+	+	+	+	+	+	+	+
Ethanol < 40% (v/v)	+	+	+	+	+	•	+	+
Benzyl alcohol < 2% (v/v)	•	+	+	+	+	+	+	+
Isopropyl alcohol < 30% (v/v)	+	+	+	+	+	+	+	+
Hydrochloric acid 0.1 M	+	•	+	+	+	+	+	+
Nitric acid 0.1 M	+	+	+	+	+	•	+	+
Phosphoric acid 25%	+	•	+	+	+	•	+	+
Sodium chloride < 2 M	+	•	+	+	+	+	+	+
Sodium hydroxide < 2 M	+	+	+	+	+	+	+	+
Triton® X-100	+	+	+	+	+	+	+	+
Guanidine HCl < 6 M	+	•	+	+	+	+	+	+
Urea < 8 M	+	+	+	+	+	+	+	+

+ Compatible • Conditional compatibility; contact Pall for details

Abbreviations: EPDM = Ethylene propylene di-monomer, FEP = Fluoroethylene polymer, PE = Polyethylene, PEEK = Polyether ether ketone, PP = Polypropylene, PTFE = Polytetrafluoroethylene, PVDF = Polyvinylidene fluoride, SS = Stainless steel

## Installation and Validation Documentation

Resolute columns are supplied with a comprehensive validation and support package including an operator's manual. The validation support package contains comprehensive protocols and provides the required documentation and procedures to support factory acceptance test (FAT), installation qualification (IQ) and operational qualification (OQ). To support validation efforts and efficient commissioning, the documentation includes records of the pre-shipment test results.

Column manual documentation includes:

- ▶ Operating guide
- ▶ Materials and compatibility data
- ▶ Comprehensive parts list
- ▶ Spare parts recommendation
- ▶ Maintenance recommendation

## Packing Station

Resolute slurry packing systems (SPS) simplify column packing and unpacking operations. Two system capacity options provide flow rates suitable for packing and unpacking columns up to 1200 mm diameter (2000 mm columns are also supported). Complete column packing solutions for Resolute columns include:

- ▶ Resolute SPS with optional control module for remote operation of slurry and buffer tank valves
- ▶ Media preparation system
- ▶ Hose kits for connection of column, system, and tanks
- ▶ Pressure and flow monitoring
- ▶ Media and buffer tanks ready for use with Resolute columns and SPS systems

## Additional Column and Equipment Needs

- ▶ Column maximum allowable pressure up to 10 barg (145 psig)
- ▶ Pressure vessel certification to ASME
- ▶ Alternative column diameters may be specified (440 and 700 mm) up to 2000 mm
- ▶ Electric actuation of adjustable end cell (not available on 280 mm column)
- ▶ Fully automated AutoPak systems for packing, unpacking, repacking and CIP
- ▶ Integrated column maintenance assist system (not available on 280 mm column)
- ▶ Automated column packing systems

**Figure 8**

*Resolute slurry packing system*



For more information on the Resolute slurry packing system, contact your local Pall representative or visit [www.pall.com/biopharm](http://www.pall.com/biopharm).

## Columns Available Within 8 Weeks Lead Time

The following standard columns are available within 8 weeks from the point of receipt of purchase order.

## Ordering Information

All columns are acrylic tubed, with manual nozzles and a stroke length of 100–400 mm. Different diameter and bed height ranges are available on request.

Part Number	Resolute Column Ø (mm)	Mesh Material	Mesh Pore Size (µm)
M-DM0301040AP10	300	PE*	10
M-DM0301040AP20			20
M-DM0301040AS10	300	SS 1.4404*	10
M-DM0301040AS20			20
M-DM0401040AP10	400	PE	10
M-DM0401040AP20			20
M-DM0401040AS10	400	SS 1.4404	10
M-DM0401040AS20			20
M-DM0451040AP10	450	PE	10
M-DM0451040AP20			20
M-DM0451040AS10	450	SS 1.4404	10
M-DM0451040AS20			20
M-DM0601040AP10	600	PE	10
M-DM0601040AP20			20
M-DM0601040AS10	600	SS 1.4404	10
M-DM0601040AS20			20
M-DM0631040AP10	630	PE	10
M-DM0631040AP20			20
M-DM0631040AS10	630	SS 1.4404	10
M-DM0631040AS20			20
M-DM0801040AP10	800	PE	10
M-DM0801040AP20			20
M-DM0801040AS10	800	SS 1.4404	10
M-DM0801040AS20			20
M-DM1001040AP10	1000	PE	10
M-DM1001040AP20			20
M-DM1001040AS10	1000	SS 1.4404	10
M-DM1001040AS20			20

\* PE = Polyethylene; SS 1.4404 = Stainless steel 1.4404

## Pressure Flow Kit (PFK)

Part Number	Applicable Column Diameter (mm)	Part Number	Applicable Column Diameter (mm)
M-PFK-030	300	M-PFK-063	630
M-PFK-040	400	M-PFK-080	800
M-PFK-045	450	M-PFK-100	1000
M-PFK-060	600		



## Column Accessories

- ▶ Endcell rotation frame for safe maintenance procedures (suitable for Resolute 400 to 1200 mm Ø columns)
- ▶ Column transportation system
- ▶ Bed support transportation device for safe and secure handling
- ▶ Column spanner set (metric)
- ▶ Packing flow kit

Contact Pall for details.

## Services

Pall has the resources necessary to help our customers maintain Pall systems and columns for optimum performance and maximum service duty. This includes comprehensive service maintenance programs, validation services, and training packages such as:

- ▶ Factory acceptance testing
- ▶ Site acceptance testing
- ▶ Installation and commissioning
- ▶ IQ/OQ documentation and execution
- ▶ Operator training (on-site or in a Pall facility)
- ▶ Planned maintenance programs
- ▶ Applications support and packing assistance
- ▶ Packing studies conducted at one of Pall's chromatography application laboratories.

Contact Pall for details.



## Chromatography Process Solutions From Pall

### Fully Automated Unit Operations with Pall Resolute Linear Columns with AutoPak Functionality

The Resolute linear column range offers a unique combination of active multi-axis piston control, precision linear actuation, and fully automated unit operations.

Building upon the proven Resolute linear column functionality, the AutoPak system is a combination of hardware and software that automates three of the key unit operations necessary in process chromatography. These key unit operations, particularly packing, can be regarded as labor intensive, high risk events. The risks include human error, packing failure and the possible absence of experienced operators which can result in plant downtime, loss of product or sorbent, and inefficient use of resources.

By adopting a full automation approach, AutoPak functionality greatly simplifies operation, effectively eliminating operator error during sequence execution and reducing the burden on key operators. The automated sequences assure that reproducible, consistent operation is the norm.

Key features of the AutoPak system:

- ▶ Ability to pack all the slurry in the tank, eliminating sorbent carry over or wastage
- ▶ Simple operator orientated operation for packing, unpacking, and CIP
- ▶ Simplified set-up, departing from traditional pump pack methods for quicker turnaround of packing and unpacking operations
- ▶ Consistent operation and reproducible results



### PK and PKP Chromatography Systems

The PK and PKP chromatography

systems range incorporates proven component technology that delivers accurate precision gradients and in-line buffer dilution. It maintains the performance necessary to make your chromatography process consistent from batch-to-batch and optimal at pilot and manufacturing scales.

Covering a range from 1 to greater than 4000 L/h, PK and PKP systems ensure robust operation and flexibility for all biochromatography applications.

These systems can be used with Resolute chromatography columns as well as Mustang® membrane adsorber products.



### Chromatography Sorbents

Pall offers a comprehensive, versatile, and environmentally friendly range of chromatography sorbents that greatly simplifies protein purification challenges from lab to process scale. Pall chromatography sorbents can improve downstream purification accuracy, speed, safety,

and reliability, and decrease purification costs. Sorbent chemistries include ion exchange, mixed-mode, affinity, hydroxyapatite and solvent detergent removal.



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#### International Offices

Pall Corporation has offices and plants throughout the world in locations such as: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, the United Kingdom, the United States, and Venezuela. Distributors in all major industrial areas of the world. To locate the Pall office or distributor nearest you, visit [www.pall.com/contact](http://www.pall.com/contact).

The information provided in this literature was reviewed for accuracy at the time of publication. Product data may be subject to change without notice. For current information consult your local Pall distributor or contact Pall directly.

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# M-7125, M-7250 Aseptic BioPharmaceutical Microfluidizer® Processor

TB-BP7.A-1

## Key features

- Up to 15 lpm (4.0 gpm) flow rates at 690 bar (10,000 psi)
- 7.5 lpm product flow (2.0 gpm) at 1,379 bar (20,000 psi)
- 4 lpm product flow (1.0 gpm) at 2,068 bar (30,000 psi)
- Low product holdup volume (<1 liter)
- Small batch capable (minimum 12 liters)
- Complete package unit including motor starter panel and process interlocks
- All product paths are sanitary grade and BPE compliant
- All instruments and valves are sanitary grade, BPE compliant
- On board data acquisition for complete batch record audit trail
- Multi-point temperature sensing for assured SIP process
- On board flow meter to measure product and CIP flow rates
- Ultra Clean In Place (UCIP) using supplied feed pump or your CIP system pump
- PID control of process chilled water for product temperature management
- Factory Acceptance Testing (FAT)
- Complete document turn over package for validation support including IQ/OQ, materials certifications and calibrations
- On site start-up assistance, operator and maintenance training, SAT and IQ/OQ execution by our technical staff



Model shown is subject to change depending on options selected

## M-7125 and M-7250 Aseptic Microfluidizer Processors Provide Superior Results For Pilot and Production Environments

### Recommended for sterile processing

- Nano-emulsions (with and without API)
- Nano-dispersions
- Microencapsulation
- Deagglomeration
- Cell disruption

### Key benefits

- Guaranteed scale up from lab and pilot Microfluidizer processors
- Validatable sterility that always passes Sterile Fill Test
- Easy to operate with simple manual controls
- Easy to maintain with most maintenance points easily accessed
- Highly secure batch records, 21 CFR Part 11 compliant
- CIP process with no equipment takedown
- Thermally sensitive materials processed safely
- More efficient processing, usually requiring fewer passes than other processing machinery
- Batch to batch process reproducibility assured

Since 1984, Microfluidics has provided life sciences and formulation scientists with critical tools used in the development and production of pharmaceutical formulations and recombinant technologies. High shear fluid processing, Microfluidics' proprietary technology, uniformly reduces droplet and particle size to enable the production of stable nano-emulsions, nano-suspensions, liposomes and the nano-encapsulation of actives. In addition it offers the most efficient method for disruption of yeast, E.coli, plant and mammalian cells.

## Discovery to Commercialization

As a result of recent advances in high throughput screening and drug discovery, many new chemical compounds have been identified as possible drug candidates. Unfortunately, many of these compounds show poor water solubility and often are only marginally soluble in oil-based solvents. The ultrahigh shear force developed by Microfluidizer processors solves this problem by reducing the particle size of active pharmaceutical ingredients to therapeutically relevant sizes that enables the production of drug products with improved bioavailability and stability.

## Cell Disruption for Biotechnology

From the gentle disruption of cultured cells for virus isolation to the challenging disruption of yeast and other fungi, Microfluidics offers technologies to meet the variable and demanding needs for cell membrane disruption. This technology provides exacting process control for highly reproducible and efficient cell breakage while keeping temperatures under precise control to prevent denaturing.

## Getting To Full Production

Results obtained on all laboratory units will scale up easily and in a linear manner to production volumes when the same operating conditions are employed. Aseptic Microfluidizer processors include Steam in Place (SIP) and Ultra Clean In Place (UCIP) eliminating the need for disassembly and Clean Out of Place (COP). Data recording and validation support documentation including IQ/OQ is included to ensure your ability to comply with 21CFR part 11 guidelines.



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[www.microfluidicscorp.com](http://www.microfluidicscorp.com)

	M-7125	M-7250
Pressure Range	Up to 689, 1379, or 2068 bar (10,000, 20,000 or 30,000 psi)	
Product Flow Rate	Up to 7.56 lpm (2.0 gpm)	Up to 15.12 lpm (4.0 gpm)
Product Feed Temperature Range	-10°C to 75°C (14°F to 165°F)	
Power Requirement	18.6 kw (25 hp)	37.3 kw (50 hp)
Utility Requirements	<ul style="list-style-type: none"> <li>Cooling water for hydraulic oil heat exchanger, preferably tower or city water</li> <li>Cooling water for product heat exchanger, preferably chilled water loop</li> <li>Compressed air for feed pump and cycling control switches requires 0.65 m<sup>3</sup>/min @ 6.2 bar (23 scfm @ 90 psi) with -37° C to -18° C (-35° F to 0° F) dew point</li> <li>Sterile steam 22.6 kg/hr @ 2.4 bar (50 lbs/hr @ 35 psi) minimum</li> <li>Sterile compressed air for product path cool/dry down, 0.085 m<sup>3</sup>/min @ 1 bar (3 scfm @ 15 psi)</li> </ul>	
Dimensions (L x W x H)*	272 x 161 x 211 cm (107" x 63.3" x 83")	
Weight with oil*		1,592 kg (3,510 lbs)

\*all weights and dimensions are approximate

## Aseptic Package Includes

- Steam In Place (SIP) with multiple RTDs to assure complete sterilization
- Ultra-Clean-In-Place (UCIP)
- Heat exchanger with product temperature control, pharma grade, double tubesheet style
- Flow meter for batch monitoring and validation
- Yokogawa data acquisition station for temperatures, pressures and flows
- Product wetted surfaces at 20Ra max
- IQ/OQ documentation and execution
- Factory Acceptance Testing (FAT) and Site Acceptance Test (SAT)
- Feed pump, sanitary cGMP grade
- 21 CFR part 11 compliant for electronic signature and batch record keeping
- Motor starter panel with machine and process interlocks
- CE compliant

## Available Options

- Motor voltage as needed
- ATEX compliant version available
- Explosion proof (XP) version available

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sartorius stedim  
biotech

## BIOSTAT<sup>®</sup> RM and Flexsafe<sup>®</sup> RM for Seed Production and Small-scale Protein Supply



turning science into solutions

# BIOSTAT<sup>®</sup> RM and Flexsafe<sup>®</sup> RM Bags

The BIOSTAT<sup>®</sup> RM, a fully GMP compliant, single-use, wave-mixed bioreactor and Flexsafe<sup>®</sup> bags are proven for a broad range of different cell lines incl. CHO, NS0, SF9, E.coli and mesenchymal stem cells.

Benefit from our excellent global application support and technical service and get started right away.



## ► Process Step

- Protein and cell supply for preclinical purposes
- Seed production for large bioreactors
- GMP production

## ► Cells

- Mammalian, insect and plant cells
- Suspension cells and adherent cells on microcarriers
- Low to medium density microbial cultures
- Shear sensitive cells such as stem cells

## ► Purpose

- Scale-up from shake or T-flasks
- Rapid material supply for pre-clinical trials
- Expansion and differentiation of stem cells
- Production of recombinant proteins, mAbs and vaccines
- Continuous cultures with reported cell densities of 150 million cells/mL

## ► Volumes

- 100 mL to 100 L culture volume
- Two rocker sizes: 20/50 and 200



► Excellent Cell Growth and Robustness

► Security of Supply & Consistent Quality

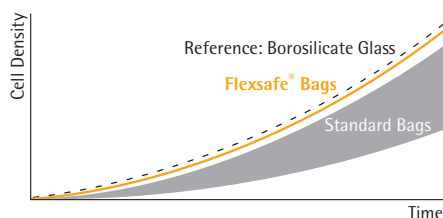
► Easy-to-use Rocker with Advanced Control Capabilities

# New Flexsafe® Bag Family

Use our Flexsafe® RM bags in your seed train and scale-up to our BIOSTAT STR® single-use stirred tank bioreactors equipped with Flexsafe STR® bags. Benefit from the same polyethylene film material across all your cell culture steps. Our Flexsafe® RM bags fit on rocking motion bioreactors from several manufacturers. Benefit from excellent cell growth, robustness and unprecedented assurance of supply.

## Cell Growth

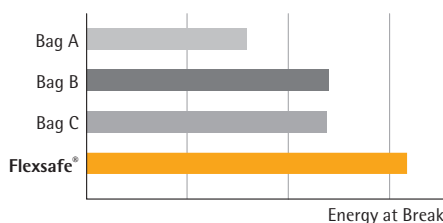
Flexsafe® ensures an excellent and reproducible growth behavior with the most sensitive production cell lines. The complete control of our raw materials, the extrusion process and the bag assembling guarantees consistent lot to lot cell growth performance.



To ensure consistent cell growth performance, we optimized the resin and minimized the additive package in collaboration with our resin and film suppliers.

**The bags are proven to be free of cytotoxic leachables by independent labs. No bDtBPP identified in WFI extract of Flexsafe® bags.**

## Robustness



The thickness, strength and flexibility of the new polyethylene film enhances the mechanical robustness of Flexsafe®, making it ideal for all bio-processing applications. The strength of Flexsafe® significantly reduces the risk of accidental damage to the bag due to inappropriate handling. Its flexibility enables convenient installation and self-deployment of the bag in its container.

## Assurance of Supply

Flexsafe® provides you with an unprecedented assurance of supply and enables robust business continuity plans. Our strategic partnership with resin and film suppliers ensures full traceability of raw materials and control over the entire manufacturing process from the resins to the final assembled bags.

### Global SUPPLY GUARANTEE

- 10-year contract with the film manufacturer
- Last time buy option for minimum of 2 years resin demand
- Up to 2 years safety stock of film
- Multiple manufacturing sites

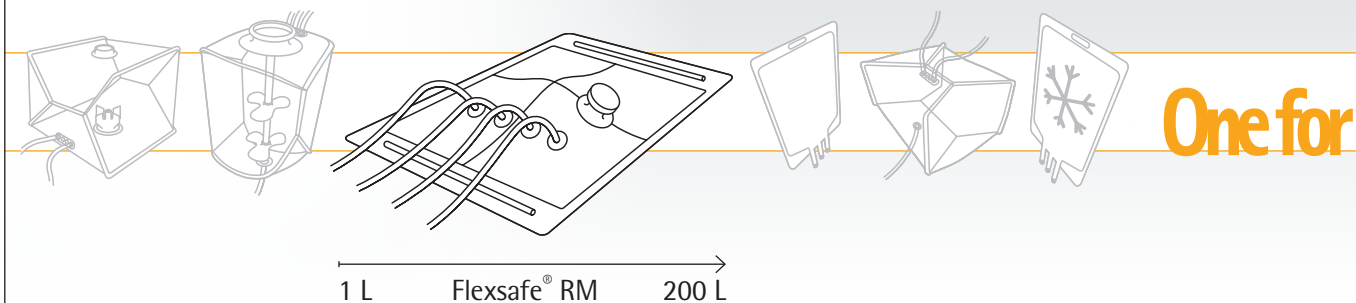
Watch Video:  
[www.sartorius.com/video-flexsafe](http://www.sartorius.com/video-flexsafe) ◀▶



New Bag Family







## Designs

The bags come in 7 different sizes supporting working volumes from 100 mL up to 100 L. Standard designs are available from stock.

	Basic Bag	Optical Bag	Perfusion Bag
For cultivation under constant conditions without pH and DO control for seed train	•		
With single-use pH and DO sensors installed in bag for advanced applications		•	•
With integrated perfusion membrane, fixed at the bottom of the bag, for continuous processing with low membrane fouling and long cultivation times			•
With single-use viable biomass sensors (BioPAT® ViaMass)	•	•	•
Available with screw caps for bulk additions	•	•	•
Customized bag designs	•	•	•
Can be used on competitor systems	•		

AI



## BIOSTAT<sup>®</sup> RM 20 | 50 Basic

The BIOSTAT<sup>®</sup> RM 20 | 50 basic is a perfectly sized, stand-alone bioreactor for bench-top use. It features an exchangeable bag holder to fit bags from 1 L to 50 L total volume. The BIOSTAT<sup>®</sup> RM basic rocker with integrated local controller, Air | CO<sub>2</sub> mixing module and load cells is the optimal choice for straight forward applications such as seed generation.

- Space-saving, individual control of two bags on the same platform
- Advanced alarming and safety features for safe cultivation
- Reduced manual handling via automated sampling function



# Advanced Control System

Do you want to run fully automated and controlled batch, fed-batch or high cell density perfusion cultures? Combine your basic rocker with our BIOSTAT® B control tower and use Flexsafe® RM bags equipped with single-use pH, DO and now also viable biomass probes.

- Advanced control of gas mixture and flow rate, filling volume and substrate addition
- Extra small space requirement with TWIN configuration – two culture systems controlled by one controller at the same time

Your BIOSTAT® B controller is designed to work with a conventional glass vessel, the single-use UniVessel® SU and the RM rocker, making it a real multi talent. It allows you to use the same controller platform for research purposes, process development and preclinical supply.

Watch Video:

[www.sartorius.com/video-biostat-b](http://www.sartorius.com/video-biostat-b) ◀▶



## **NEW** BioPAT® ViaMass\* – single-use biomass sensor

for non-invasive measurement of viable biomass. BIOSTAT® RM is the only rocking motion bioreactor with inline single-use biomass monitoring.

- Reduce your contamination risk by using inline single use cell growth monitoring, e.g. in cell therapy or other critical applications



\* Manufactured under license from HAMILTON.

# BIOSTAT<sup>®</sup> B with RM 200 Rocker

The system consists of the BIOSTAT<sup>®</sup> RM 200 Rocker, BIOSTAT<sup>®</sup> B control tower on lab-cart and Flexsafe<sup>®</sup> RM cultivation bag. It is your choice for larger scale seed expansion and production of material for preclinical and clinical studies. It supports working volumes from 10 L up to 100 L. Together with our proven BIOSTAT<sup>®</sup> B control tower, flexible process strategies can be enabled.

- Low consumable costs compared to stirred single-use bioreactors
- Reliable single-use probes for measurement of pH, DO and viable biomass
- Designed for automated batch, fed-batch and perfusion processes using an external cell retention unit
- Flexible arrangement of control tower and rocker unit on individual trolleys





# Technical Specifications

## Flexsafe® RM Bags

Total Volume (L)	1	2	10	20	50	100	200
Working volume (L)*	0.1–0.5	0.2–1	1–5	2–10	5–25	10–50	20–100
Bag Types							
Basic	•	•	•	•	•	•	•
Basic and optical with screw cap		•	•	•	•		
Optical		•	•	•	•	•	•
Perfusion with integrated membrane		•	•	•	•		
Perfusion with connection to ATF** System		•	•	•	•	•	•
Basic, optical, perfusion & perfusion ATF with ViaMass			•	•	•		
Main Components	Basic		Optical		Perfusion		
Connectors	Female   Male Luer Female   Male MPC		Female   Male Luer Female   Male MPC		Female   Male Luer Female   Male MPC		
Filters	Air Inlet   Air Outlet		Air Inlet   Air Outlet		Air Inlet   Air Outlet		
Ports	Sampling Fill   harvest with dip tube Acid   Base		Sampling Fill   harvest with dip tube Acid   Base		Sampling Fill   harvest with dip tube Acid   Base Perfusion feed   harvest		
Sensors	–		Optical chemical pH Optical chemical DO Single-use viable biomass		Optical chemical pH Optical chemical DO Single-use viable biomass		
Perfusion	–		–		Perfusion membrane PES 1.2 µm   Opta aseptic connector to ATF** System		
Tubing material	C-Flex <sup>®</sup> 374, Silicone, PharMed <sup>®</sup> Natvar ABL-1222 TPE						
Material of the bag	Contact layer: LLDPE   gas barrier: EVOH   outer layer: LLDPE						
Production conditions	Cleanroom environment of at least ISO 8						
Biological reactivity	USP for plastics Class VI						
Sterility	ISO 11137 – sterility assurance level (SAL) of 10 <sup>-6</sup>						
TSE/BSE status	Compliant to EMA410/01/rev.3 guideline						
Endotoxin	USP<85> for sterile water for injection; < 0.25 EU/ml						
Irradiated	25 – 40 kGy						

\* Bags with sensors might require higher minimum working volumes depending on rocking rate and angle.

() – optional, needs to be ordered separately

\*\* ATF cell retention system from Repligen

\*\*\* C-Flex® and PharMed® are registered trademarks of Saint-Gobain Performance Plastics Corporation.

## Ordering Information

Flexsafe® RM	Basic Basic ViaMass	Basic screw cap Optical screw cap	Optical Optical ViaMass	Perfusion Perfusion ViaMass	Perfusion ATF Perfusion ATF ViaMass
1 L	DFB001L –	– –	– –	– –	– –
2 L	DFB002L –	DFB002L----01SC DFO002L----01SC	DFO002L –	DFP002L--SM –	DFP002L--AT –
10 L	DFB010L DFB010L----VM	DFB010L----01SC DFO010L----01SC	DFO010L DFO010L----VM	DFP010L--SM DFP010L--SMVM	DFP010L--AT DFP010L--ATVM
20 L	DFB020L DFB020L----VM	DFB020L----01SC DFO020L----01SC	DFO020L DFO020L----VM	DFP020L--SM DFP020L--SMVM	DFP020L--AT DFP020L--ATVM
50 L	DFB050L DFB050L----VM	DFB050L----01SC DFO050L----01SC	DFO050L DFO050L----VM	DFP050L--SM DFP050L--SMVM	DFP050L--AT DFP050L--ATVM
50 L *	DFB050L----01US	–	–	–	–
100 L	DFB100L –	– –	DFO100L –	– –	DFP100L--AT –
200 L	DFB200L –	– –	DFO200L –	– –	DFP200L--AT –

\* alternative dimension – 740 × 720 mm – suitable for competitor rockers

# Technical Specifications

## Facility and Utility Requirements

	BIOSTAT® RM 20   50 Basic	BIOSTAT® B with RM 20   50 Rocker	BIOSTAT® B with RM 200 Rocker
<b>Power Supply (Country Specific)   Frequency   Electricity   Consumption   Protection Class</b>			
Rocker platform	100 (240 V)   60 (50) Hz   600 W   IP23	100 (240 V)   60 (50) Hz   600 W   IP23	120 (230 V)   60 (50) Hz   10 (5.2) A   1200 W   IP21
Control Tower	–	230 V   50 Hz   10 A   IP21 or 120 V   60 Hz   12 A   IP21	230 V   50 Hz   10 A   IP21 or 120 V   60 Hz   12 A   IP21
Load cells	100 – 240 V   15 W	100 – 240 V   15 W	
<b>Gas Supply</b>			
Inlet pressure (barg)	1.0 – 1.5	1.0 – 1.5	1.0 – 1.5
Quick coupling for gas tubes, Festo Type	Ø 4 mm	(Ø 6 mm)	(Ø 6 mm)
Connection hose coupling, external	–	Ø 6 mm	Ø 6 mm
<b>Gas Specification According to ISO 8573-1: dry, free of oil and dust</b>			
Particle size: <0.1 mm	•	•	•
Max. amount 0.1 mg/m <sup>3</sup> (class 1)	•	•	•
Condensate: dew point < 3 °C (class 4)	•	•	•
Oil <0.01 mg/m <sup>3</sup> (class 1)	•	•	•
Germs (class 0)	•	•	•
<b>Water</b>			
Water supply pressure (barg)	–	2 – 8	2 – 8
Connection hose coupling, external	–	Ø 10 mm	Ø 10 mm
Cooling water (for heating   cooling system only)	–	(•)	(•)
Temperature	–	min. 4°	min. 4°
Degree of hardness	–	max. 12 dH	max. 12 dH
<b>Operative Environment</b>			
Ambient temperature	5–40 °C		
Relative humidity range	50% (40 °C) – 80% (31 °C)		

## System Characteristics

	Dimensions W×D×H	Weight	Material
BIOSTAT® B Control Tower Single   Twin	410×520×810 mm 16×20×32 in	40   55 kg 88   121 lbs	Stainless steel AISI 304
BIOSTAT® RM 20 Rocker complete	765×600×510 mm 30×24×16 in	30 kg 66 lb	Stainless steel, ABS
Bag holder 20	765×600×60 mm 30×24×2.4 in	5.5 kg 12 lb	Stainless steel, ABS
Lid 20	765×600×255 mm 30×24×10 in	2.5 kg 5.5 lb	ABS
BIOSTAT® RM 50 Rocker complete	1085×600×510 mm 43×24×18 in	31.3 kg 69 lb	Stainless steel, ABS
Bag holder 50	1085×600×60 mm 43×24×2.4 in	7.8 kg 17 lb	Stainless steel, ABS
Lid 50	1085×600×255 mm 43×24×10 in	3.7 kg 8 lb	ABS
Load cells for BIOSTAT® RM 20   50	609×536×60-68 mm 24×21×2.4-2.7 in	9 kg 20 lb	Stainless steel, ABS
BIOSTAT® RM 200 Rocker in 45° transport position	1940×905×1285 mm 77×36×51 in	197 kg 434 lb	Stainless steel, ABS
BIOSTAT® RM 200 Rocker in horizontal position	1940×1080×1155 mm 76×43×46 in	197 kg 434 lb	Stainless steel, ABS
Lab-cart	800×800×900 mm 32×32×36 in	88 kg 194 lb	Stainless steel



## Process Control

	BIOSTAT® RM 20   50 Basic	BIOSTAT® B with RM 20   50 Rocker	BIOSTAT® B with RM 200 Rocker
<b>Temperature Module</b>			
<b>Heating Only – Electrical Heating Plates</b>			
Temperature control	RT–40°C	RT–40°C	RT–40°C
Heating capacity	2 × 140 W (48 V)	2 × 140 W (48 V)	2 × 410 W
<b>Heating   Cooling (Option) – Open Thermostat System with Circulation Pump and Automatic Cooling Water Valve</b>			
Temperature control	–	8°C above cooling water up to 40°C	(8°C above cooling water up to 40°C)
Heating capacity	–	1 × 600 W	2 × 600 W
Over temperature protection	•	•	•
<b>Gassing Module Rocker – Optional</b>			
Max. total flow (ml/min) Controlled by MFC	One bag: 1 × 1 lpm Twin bag: 2 × 0.5 lpm	–	–
Fixed CO <sub>2</sub> gassing (%) measured by IR sensor	0.8 – 15 ± 5 %	–	–
Internal air pump	(•)	–	–
<b>Gassing Module Control Tower</b>	4-Gas mix (O <sub>2</sub> , N <sub>2</sub> , CO <sub>2</sub> , air) with headspace outlet		
Rotameters	–	max. 4	max. 4
– flow rates		0.016 lpm – 8.3 lpm	0.016 lpm – 13 lpm
– accuracy		± 5% full scale	± 5% full scale
MFC	–	max. 4	max. 4
– flow rates		0.003 lpm – 5 lpm	0.06 lpm – 10 lpm
– accuracy		± 1% full scale	± 1% full scale
4-stage DO cascade	–	•	•
Advanced DO controller	–	(•)	(•)
<b>Sensors &amp; Measurement</b>			
Temperature probe Pt 100	•	•	•
– temperature range	0 – 150°C	0 – 150°C	0 – 150°C
– display resolution	0.1°C	0.1°C	0.1°C
– amplifiers	2	1 (single)   2 (twin)	2
pH single use	–	•	•
– measurement range		6.5–8.5	6.5–8.5
– display resolution		0.1 pH	0.1 pH
– amplifiers		1 (single)   2 (twin)	2
– recalibration function		•	•
DO single-use	–	•	•
– measurement range		0 – 250%	0 – 250%
– display resolution		0.1%	0.1%
– amplifiers		1 (single)   2 (twin)	2
– recalibration function		•	•
Single-use viable biomass (BioPAT® ViaMass)	–	(•)	(•)
Load cells	(•)	(•)	(•)
– weight range   accuracy	max. 30 kg   10 g	max. 30 kg   10 g	max. 120 kg   100 g
Balance substrate	–	(up to 2) per side	(up to 2) per side
External signal input	–	max. 2 0–10 V or 4–20 mA	2

() – optional, needs to be ordered separately



Pump Module	
BIOSTAT® B with RM 20   50 Rocker and BIOSTAT® B with RM 200 Rocker	
max. 4 internal and 2 external pumps, thereof 3 speed controlled per side	
Built-in Pumps	
<b>Fixed Speed</b>	Watson Marlow 114, Fast Load pump head
– Speed 5 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0–0.1 ml/min ID: 0.8 mm: 0.05–2.4 ml/min ID: 1.6 mm: 0.01–0.7 ml/min ID: 2.4 mm: 0.03–1.5 ml/min ID: 3.2 mm: 0.05–2.4 ml/min ID: 4.8 mm: 0.09–4.3 ml/min
– Speed 44 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0.02–0.9 ml/min ID: 0.8 mm: 0.04–1.8 ml/min ID: 1.6 mm: 0.12–6.2 ml/min ID: 2.4 mm: 0.26–12.8 ml/min ID: 3.2 mm: 0.41–20.7 ml/min ID: 4.8 mm: 0.75–37.4 ml/min
<b>Speed Controlled</b>	Watson Marlow 114, Fast Load pump head
– Speed 0.15–5 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0–0.1 ml/min ID: 0.8 mm: 0.01–0.2 ml/min ID: 1.6 mm: 0.02–0.7 ml/min ID: 2.4 mm: 0.04–1.5 ml/min ID: 3.2 mm: 0.07–2.4 ml/min ID: 4.8 mm: 0.13–4.3 ml/min
– Speed 5–150 rpm Flow rate (tubing wall thickness 1.6 mm)	ID: 0.5 mm: 0.1–3 ml/min ID: 0.8 mm: 0.2–6 ml/min ID: 1.6 mm: 0.7–21 ml/min ID: 2.4 mm: 1.45–43.5 ml/min ID: 3.2 mm: 2.35–70.5 ml/min ID: 4.8 mm: 4.25–127.5 ml/min
External Pumps	
<b>Speed Controlled</b>	Watson Marlow 520, Fast Load pump head, up to 200 rpm Watson Marlow 323



# Communication

	BIOSTAT® RM 20   50 Basic	BIOSTAT® B with RM 20   50 Rocker	BIOSTAT® B with RM 200 Rocker
Industrial Ethernet	1	1	1
Profibus DP	(1)	–	–

The BIOSTAT® RM rocker is designed to communicate with with industrial SCADA or DCS systems (e.g. DeltaV) through the standard Modbus RTU or an optional Profibus DP interfaces.



## Technical Data

	BIOSTAT® RM 20   50 Basic	BIOSTAT® B with RM 20   50 Rocker	BIOSTAT® B with RM 200 Rocker
Max. total volume (L)	50	50	200
Working volume (L)	0.1–25	0.1–25	10–100
Rocking rate (r/min)	8–42 ± 1	8–42 ± 1	2–20 ± 1
Rocking angle (°)	4–10 ± 0.3	4–10 ± 0.3	2–10 ± 0.3
Clamping rails for bag fixation	•	•	•
Sensor clamps for secure fixation of glass fiber cables	–	2 (single)   4 (twin)	4
Filter heater	2	1 (single)   2 (twin)	2
Integrated Rocker Controller	•	•	–
BIOSTAT® B Control Tower	–	•	•
Color touch screen	•	•	•
Potential-free alarm contact	• (max. 0.5 A)	•	•
Safety measurement and shut-off	30 mbar (in combination with gassing module)	30 mbar	30 mbar
Additional safety valve gasses (mbar)	–	100 mbar	100 mbar
Water inlet pressure reduction value	–	1.5 bar, integrated pressure control	1.5 bar, integrated pressure control
Different user level log in	•	(•)	(•)
Logbook function	–	(•)	(•)
Lab-cart for BIOSTAT® B Control Tower	–	–	•



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# Chapter 1

## Dimensional Analysis

### 1.1 Introduction

Before beginning the material on dimensional analysis, it is worth considering a simple example that demonstrates what we are doing. One that qualifies as simple is the situation of when an object is thrown upwards. The resulting mathematical model for this is an equation for the height  $x(t)$  of the projectile from the surface of the Earth at time  $t$ . This equation is determined using Newton's second law,  $F = ma$ , and the law of gravitation. The result is

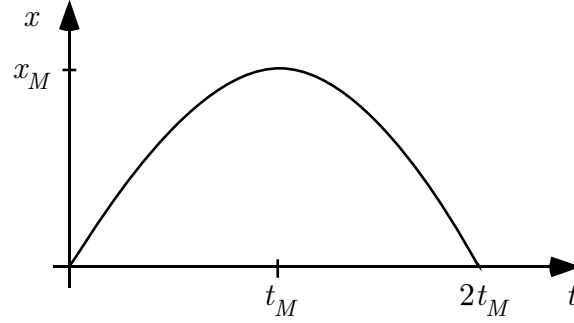
$$\frac{d^2x}{dt^2} = -\frac{gR^2}{(R+x)^2}, \quad \text{for } 0 < t, \quad (1.1)$$

where  $g$  is the gravitational acceleration constant and  $R$  is the radius of the Earth. Finding the solution  $x$  of this equation requires two integrations. Each will produce an integration constant, and we need more information to find these constants. This is done by specifying the initial conditions. Assuming the projectile starts at the surface with velocity  $v_0$  then the initial conditions are as follows

$$x(0) = 0, \quad (1.2)$$

$$\frac{dx}{dt}(0) = v_0. \quad (1.3)$$

The resulting initial value problem for  $x$  consists in finding the solution of (1.1) that satisfies (1.2) and (1.3). Mathematically, the problem is challenging because it involves solving a second-order nonlinear differential equation. One option for finding the solution is simply to use a computer. However, the limitation with this is that it does not provide much insight into how the solution depends on the terms in the equation. One of the primary objectives of this text is to use mathematics to derive a fundamental understanding of how and why things work the way they do, and so, we are very interested in



**Figure 1.1** The solution (1.5) of the projectile problem in a uniform gravitational field.

obtaining at least an approximate solution of this problem. This is the same point-of-view taken in most physics books and it is worth looking at how they might address this issue. Adopting for the moment the typical Physics I approach, in looking at the equation in (1.1) it is not unreasonable to assume  $R$  is significantly larger than even the largest value of  $x$ . If true then we should be able to replace the  $x+R$  term with just  $R$ . In this case the problem reduces to solving

$$\frac{d^2x}{dt^2} = -g, \quad \text{for } 0 < t. \quad (1.4)$$

Integrating and then using the two initial conditions yields

$$x(t) = -\frac{1}{2}gt^2 + v_0t. \quad (1.5)$$

This solution is shown schematically in Figure 1.1. We have what we wanted, a relatively simple expression that serves as an approximation to the original nonlinear problem. To complete the derivation we should check that the assumption made in the derivation is satisfied, namely  $x$  is much smaller than  $R$ . Now, the maximum height for (1.5) occurs when

$$\frac{dx}{dt} = 0. \quad (1.6)$$

Solving this equation yields  $t = v_0/g$  and from this it follows that the maximum height is

$$x_M = \frac{v_0^2}{2g}. \quad (1.7)$$

Therefore, we must require that  $v^2/(2g)$  is much less than  $R$ , which we write as  $v_0^2/(2g) \ll R$ .

It is now time to critique the above derivation. The first criticism is that the approach is heuristic. The reason is that even though the argument for replacing  $x+R$  with  $R$  seems plausible, we simply ignored a particular term in the equation. The projectile problem is not particularly complicated so

dropping a term as we did is straightforward. However, in the real world where problems can be quite complicated, dropping a term in one part of the problem can lead to inconsistencies in another part. A second criticism can be made by asking a question. Specifically, what exactly is the effect of the nonlinearity on the projectile? Our reduction replaced the nonlinear gravitational force, which is the right-hand side of (1.1), with a uniform gravitational field given by  $-g$ . Presumably if gravity decreases with height then the projectile will be going higher than we would expect based on our approximation in (1.5). It is of interest to understand quantitatively what this nonlinear effect is and whether it might interfere with our reduction.

Based on the comments of the previous paragraph we need to make the reduction process more systematic. The procedure that is used to simplify the problem should enable us to know exactly what is large or small in the problem, and it should also enable us to construct increasingly more accurate approximations to the problem. Explaining what is involved in a systematic reduction occurs in two steps. The first, which is the objective of this chapter, involves the study of dimensions and how these can be used to simplify the mathematical formulation of the problem. After this, in Chapter 2, we develop techniques to construct accurate approximations of the resulting equations.

## 1.2 Examples of Dimensional Reduction

The first idea that we explore will, on the surface, seem to be rather simple, but it is actually quite profound. It has to do with the dimensions of the physical variables, or parameters, in a problem. To illustrate, suppose we know that the speed  $s$  of a ball is determined by its radius  $r$  and the length of time  $t$  it has been moving. Implicit in this statement is the assumption that the speed does not depend on any other physical variable. In mathematical terms we have that  $s = f(r, t)$ . The function  $f$  is not specified and all we know is that there is some expression that connects the speed with  $r$  and  $t$ . The only possible way to combine these two quantities to produce the dimension of speed is through their ratio  $r/t$ . For example, it is impossible to have  $s = \alpha r + \beta t$  without  $\alpha$  and  $\beta$  having dimensions. This would mean  $\alpha$  and  $\beta$  are physical parameters, and we have assumed there are no others in the problem. This observation enables us to conclude that based on the original assumptions that the only function we can have is  $s = \alpha r/t$ , where  $\alpha$  is a number.

What we are seeing in this example is that the dimensions of the variables in the problem end up dictating the form of the function. This is very useful information and we will spend some time exploring how to exploit this idea. To set the stage we need to introduce some of the terminology. The first is the concept of a fundamental dimension. As is well known, physical variables such as force, density, and velocity can be broken down into length  $L$ , time

Quantity	Dimensions	Quantity	Dimensions
Acceleration	$LT^{-2}$	Enthalpy	$ML^2T^{-2}$
Angle	1	Entropy	$ML^2T^{-2}\theta^{-1}$
Angular Acceleration	$T^{-2}$	Gas Constant	$L^2T^{-2}\theta^{-1}$
Angular Momentum	$ML^2T^{-1}$	Internal Energy	$ML^2T^{-2}$
Angular Velocity	$T^{-1}$	Specific Heat	$L^2T^{-2}\theta^{-1}$
Area	$L^2$	Temperature	$\theta$
Energy, Work	$ML^2T^{-2}$	Thermal Conductivity	$MLT^{-3}\theta^{-1}$
Force	$MLT^{-2}$	Thermal Diffusivity	$L^2T^{-1}$
Frequency	$T^{-1}$	Heat Transfer Coefficient	$MT^{-3}\theta^{-1}$
Concentration	$L^{-3}$		
Length	$L$	Capacitance	$M^{-1}L^{-2}T^4I^2$
Mass	$M$	Charge	TI
Mass Density	$ML^{-3}$	Charge Density	$L^{-3}TI$
Momentum	$MLT^{-1}$	Conductivity	$M^{-1}L^{-3}T^3I^2$
Power	$ML^2T^{-3}$	Electric Current Density	$L^{-2}I$
Pressure, Stress, Elastic Modulus	$ML^{-1}T^{-2}$	Electric Current	$I$
Surface Tension	$MT^{-2}$	Electric Displacement	$L^{-2}TI$
Time	$T$	Electric Potential	$ML^2T^{-3}I^{-1}$
Torque	$ML^2T^{-2}$	Electric Field Intensity	$MLT^{-3}I^{-1}$
Velocity	$LT^{-1}$	Inductance	$ML^2T^{-2}I^{-2}$
Viscosity (Dynamic)	$ML^{-1}T^{-1}$	Magnetic Field Intensity	$L^{-1}I$
Viscosity (Kinematic)	$L^2T^{-1}$	Magnetic flux	$L^2MT^{-2}I^{-1}$
Volume	$L^3$	Permeability	$MLT^{-2}I^{-2}$
Wave Length	$L$	Permittivity	$M^{-1}L^{-3}T^4I^2$
Strain	1	Electric Resistance	$ML^2T^{-3}I^{-2}$

**Table 1.1** Fundamental dimensions for commonly occurring quantities. A quantity with a one in the dimensions column is dimensionless.

$T$ , and mass  $M$  (see Table 1.1). Moreover, length, time, and mass are independent in the sense that one of them cannot be written in terms of the other two. For these two reasons we will consider  $L$ ,  $T$ , and  $M$  as *fundamental dimensions*. For problems involving thermodynamics we will expand this list to include temperature ( $\theta$ ) and for electrical problems we add current ( $I$ ). In conjunction with this, given a physical variable  $x$  we will designate the fundamental dimensions of  $x$  using the notation  $[x]$ . For example,  $[velocity] = L/T$ ,  $[force] = ML/T^2$ ,  $[g] = L/T^2$ , and  $[density] = M/L^3$ .



It is important to understand that nothing is being assumed about which specific system of units is used to determine the values of the variables or parameters. Dimensional analysis requires that the equations be independent of the system of units. For example, both Newton's law  $F = ma$  and the differential equation (1.1) do not depend on the specific system one selects. For this reason these equations are said to be *dimensionally homogeneous*. If one were to specialize (1.1) to SI units and set  $R = 6378\text{ km}$  and  $g = 9.8\text{ m/sec}^2$  they would end up with an equation that is not dimensionally homogeneous.

### 1.2.1 Maximum Height of a Projectile

The process of dimensional reduction will be explained by applying it to the projectile problem. To set the stage, suppose we are interested in the maximum height  $x_M$  of the projectile. Based on Newton's second law, and the initial conditions in (1.2) and (1.3), it is assumed that the only physical parameters that  $x_M$  depends on are  $g$ ,  $v_0$ , and the mass  $m$  of the projectile. Mathematically this assumption is written as  $x_M = f(g, m, v_0)$ . The function  $f$  is unknown but we are going to see if the dimensions can be used to simplify the expression. The only way to combine  $g$ ,  $m$ ,  $v_0$  to produce the correct dimensions is through a product or ratio. So, our start-off hypothesis is that there are numbers  $a$ ,  $b$ ,  $c$  so that

$$[x_M] = [m^a v_0^b g^c]. \quad (1.8)$$

Using the fundamental dimensions for these variables the above equation is equivalent to

$$\begin{aligned} L &= M^a (L/T)^b (L/T^2)^c \\ &= M^a L^{b+c} T^{-b-2c}. \end{aligned} \quad (1.9)$$

Equating the exponents of the respective terms in this equation we conclude

$$\begin{aligned} L : \quad & b + c = 1, \\ T : \quad & -b - 2c = 0, \\ M : \quad & a = 0. \end{aligned}$$

Solving these equations we obtain  $a = 0$ ,  $b = 2$ , and  $c = -1$ . This means the only way to produce the dimensions of length using  $m$ ,  $v_0$ ,  $g$  is through the ratio  $v_0^2/g$ . Given our start-off assumption (1.8), we conclude that  $x_M$  is proportional to  $v_0^2/g$ . In other words, the original assumption that  $x_M = f(g, m, v_0)$  dimensionally reduces to the expression

$$x_M = \alpha \frac{v_0^2}{g}, \quad (1.10)$$

where  $\alpha$  is an arbitrary number. With (1.10) we have come close to obtaining our earlier result (1.7) and have done so without solving a differential equation or using calculus to find the maximum value. Based on this rather minimal effort we can make the following observations:

- If the initial velocity is increased by a factor of 2 then the maximum height will increase by a factor of 4. This observation offers an easy method for experimentally checking on whether the original modeling assumptions are correct.
- The constant  $\alpha$  can be determined by running one experiment. Namely, for a given initial velocity  $v_0 = \bar{v}_0$  we measure the maximum height  $x_M = \bar{x}_M$ . With these known values,  $\alpha = g\bar{x}_M/\bar{v}_0^2$ . Once this is done, the formula in (1.10) can be used to determine  $x_M$  for any  $v_0$ .

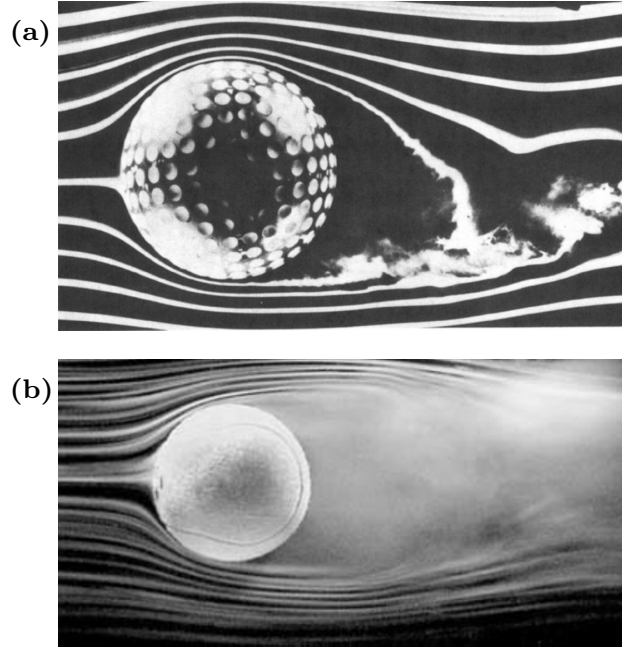
The steps we have used are the basis for the method of dimensional reduction, where an expression is simplified based on the fundamental dimensions of the quantities involved. Given how easy it was to obtain (1.10) the method is very attractive as an analysis tool. It does have limitations and one is that we do not know the value of the number  $\alpha$ . It also requires us to be able to identify at the beginning what parameters are needed. The importance of this and how this relies on understanding the physical laws underlying the problem will be discussed later.

The purpose of the above example is to introduce the idea of dimensional reduction. What it does not show is how to handle problems with several parameters and this is the purpose of the next two examples.

### 1.2.2 Drag on a Sphere

In the design of automobiles, racing bicycles, and aircraft there is an overall objective to keep the drag on the object as small as possible. It is interesting to see what insight dimensional analysis might provide in such a situation, but since we are beginners it will be assumed the object is very simple and is a sphere (see Figure 1.2). The modeling assumption that is made is that the drag force  $D_F$  on the sphere depends on the radius  $R$  of the sphere, the velocity  $v$  of the sphere, the density  $\rho$  of the air, and the dynamic viscosity  $\mu$  of the air. The latter is a measure of the resistance force of the air to motion and we will investigate this in Chapter 8. For the moment all we need is its fundamental dimensions and these are given in Table 1.1. In mathematical terms the modeling assumption is

$$D_F = f(R, v, \rho, \mu), \quad (1.11)$$



**Figure 1.2** Air flow around an object can be visualized using smoke. The flow around a golf ball is shown in (a) (Brown [1971]), and around a tennis ball in (b) (Bluck [2000]). In both cases the air is moving from left to right.

and we want to use dimensional reduction to find a simplified version of this expression. Similar to the last example, the first question is whether we can find numbers  $a$ ,  $b$ ,  $c$ ,  $d$  so that

$$[D_F] = [R^a v^b \rho^c \mu^d]. \quad (1.12)$$

Expressing these using fundamental dimensions yields

$$\begin{aligned} M L T^{-2} &= L^a (L/T)^b (M/L^3)^c (M/LT)^d \\ &= L^{a+b-3c-d} T^{-b-d} M^{c+d}. \end{aligned}$$

As before we equate the respective terms and conclude

$$\begin{aligned} L : \quad & a + b - 3c - d = 1, \\ T : \quad & -b - d = -2, \\ M : \quad & c + d = 1. \end{aligned} \quad (1.13)$$

We have four unknowns and three equations, so it is anticipated that in solving the above system of equations one of the unknowns will be undetermined. From the  $T$  equation we have  $b = 2 - d$ , and from the  $M$  equation  $c = 1 - d$ . The  $L$  equation then gives us  $a = 2 - d$ . With these solutions, and based on our assumption in (1.12), we have that

$$\begin{aligned}
D_F &= \alpha \rho R^{2-d} v^{2-d} \rho^{1-d} \mu^d \\
&= \alpha \rho R^2 v^2 \left( \frac{\mu}{R v \rho} \right)^d,
\end{aligned}$$

where  $\alpha$  is an arbitrary number. This can be written as

$$D_F = \alpha \rho R^2 v^2 \Pi^d, \quad (1.14)$$

where

$$\Pi = \frac{\mu}{R v \rho}, \quad (1.15)$$

and  $d$ ,  $\alpha$  are arbitrary numbers. This is the *general product solution* for how  $D_F$  depends on the given variables. The quantity  $\Pi$  is dimensionless, and it is an example of what is known as a dimensionless product. Physically, it can be thought of as the ratio of the viscous force ( $\mu$ ) to the inertial force ( $R v \rho$ ) in the air. Calling it a product is a bit misleading as  $\Pi$  involves both multiplications and divisions. Some avoid this by calling it a dimensionless group. We will use both expressions in this book.

The formula for  $D_F$  in (1.14) is not the final answer. What remains is to determine the consequence of the arbitrary exponent  $d$ . The key observation is that given any two sets of values for  $(\alpha, d)$ , say  $(\alpha_1, d_1)$  and  $(\alpha_2, d_2)$ , then

$$\begin{aligned}
D_F &= \alpha_1 \rho R^2 v^2 \Pi^{d_1} + \alpha_2 \rho R^2 v^2 \Pi^{d_2} \\
&= \rho R^2 v^2 (\alpha_1 \Pi^{d_1} + \alpha_2 \Pi^{d_2})
\end{aligned}$$

is also a solution. Extending this observation we conclude that another solution is

$$D_F = \rho R^2 v^2 (\alpha_1 \Pi^{d_1} + \alpha_2 \Pi^{d_2} + \alpha_3 \Pi^{d_3} + \dots), \quad (1.16)$$

where  $d_1, d_2, d_3, \dots$  are arbitrary numbers as are the coefficients  $\alpha_1, \alpha_2, \alpha_3, \dots$ . To express this in a more compact form, note that the expression within the parentheses in (1.16) is simply a function of  $\Pi$ . From this observation we obtain the *general solution*, which is

$$D_F = \rho R^2 v^2 F(\Pi), \quad (1.17)$$

where  $F$  is an arbitrary function of the dimensionless product  $\Pi$ . We have, therefore, been able to use dimensional analysis to reduce (1.11), which involves an unknown of four variables, down to an unknown function of one variable. Although this is a significant improvement, the result is perhaps not as satisfying as the one obtained for the projectile example, given in (1.10), because we have not been able to determine  $F$ . However, there are various ways to address this issue, and some of them will be considered below.

## Representation of Solution

Now that the derivation is complete a few comments are in order. First, it is possible for two people to go through the above steps and come to what looks to be very different conclusions. For example, the general solution can also be written as

$$D_F = \frac{\mu^2}{\rho} H(\Pi), \quad (1.18)$$

where  $H$  is an arbitrary function of  $\Pi$ . The proof that this is equivalent to (1.17) comes from the requirement that the two expressions must produce the same result. In other words, it is required that

$$\frac{\mu^2}{\rho} H(\Pi) = \rho R^2 v^2 F(\Pi).$$

Solving this for  $H$  yields

$$H(\Pi) = \frac{1}{\Pi^2} F(\Pi).$$

The fact that the right-hand side of the above equation only depends on  $\Pi$  shows that (1.18) is equivalent to (1.17). As an example, if  $F(\Pi) = \Pi$  in (1.17), then  $H(\Pi) = 1/\Pi$  in (1.18).

Another representation for the general solution is

$$D_F = \rho R^2 v^2 G(Re), \quad (1.19)$$

where

$$Re = \frac{Rv\rho}{\mu}, \quad (1.20)$$

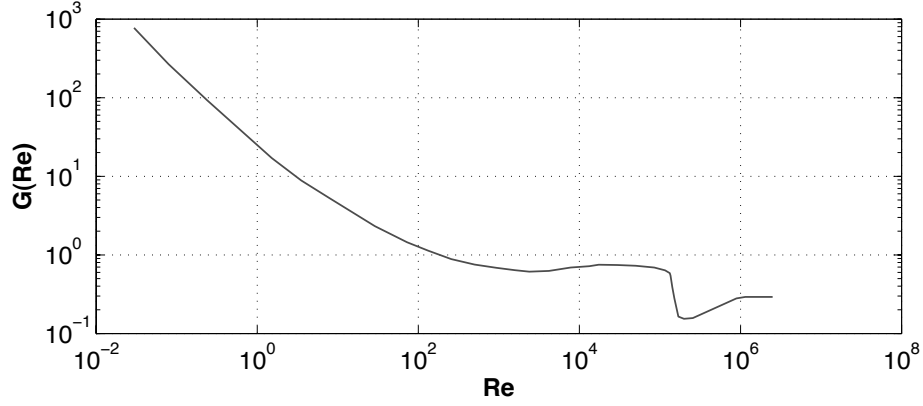
and  $G$  is an arbitrary function of  $Re$ . This form is the one usually used in fluid dynamics, where the dimensionless product  $Re$  is known as the Reynolds number. The function  $G$  is related to the drag coefficient  $C_D$ , through the equation  $G = \frac{\pi}{2} C_D$ . Because of its importance in fluids,  $G$  has been measured for a wide range of Reynolds numbers, producing the curve shown in Figure 1.3. To transform between the representation in (1.19), and the one in (1.17), note  $Re = 1/\Pi$ . From the requirement

$$\rho R^2 v^2 G(Re) = \rho R^2 v^2 F(\Pi),$$

we obtain

$$G(Re) = F(1/Re).$$

The reason for the different representations is that there are four unknowns in (1.12) yet only three equations. This means one of the unknowns is used in the general solution and, as expressed in (1.14), we used  $d$ . If you were to use one of the others then a different looking, but mathematically equivalent,



**Figure 1.3** The measured values of the function  $G(Re)$  that arises in the formula for the drag on a sphere, as given in (1.19).

expression would be obtained. The fact that there are multiple ways to express the solution can be used to advantage. For example, if one is interested in the value of  $D_F$  for small values of the velocity then (1.19) would be a bit easier to use. The reason is that to investigate the case of small  $v$  it is somewhat easier to determine what happens to  $G$  for  $Re$  near zero than to expand  $F$  for large values of  $Re$ . For the same reason, (1.17) is easier to work with for studying large velocities. One last comment to make is that even though there are choices on the form of the general solution, they all have exactly the same number of dimensionless products.

### Determining $F$

A more challenging question concerns how to determine the function  $F$  in (1.17). The mathematical approach would be to solve the equations for fluid flow around a sphere and from this find  $F$ . This is an intriguing idea and one that will be used from time to time in this book. There is, however, another more applied approach that makes direct use of (1.17). Specifically, a sequence of experiments is run to measure  $F(r)$  for  $0 < r < \infty$ . To do this a sphere with a given radius  $R_0$ , and a fluid with known density  $\rho_0$  and viscosity  $\mu_0$ , are selected. In this case (1.17) can be written as

$$F(r) = \frac{\gamma D_F}{v^2} \quad (1.21)$$

where  $\gamma = 1/(\rho_0 R_0^2)$  is known and fixed. The experiment consists of taking various values of  $v$  and then measuring the resulting drag force  $D_F$  on the sphere. To illustrate, suppose our choice for the sphere and fluid give  $R = 1$ ,  $\rho_0 = 2$ , and  $\mu_0 = 3$ . Also, suppose that running the experiment using  $v = 4$  produces a measured drag of  $D_F = 5$ . In this case  $r = \mu_0/(R_0 v \rho_0) = 3/8$  and  $\gamma D_F/v^2 = 5/32$ . Our conclusion is therefore that  $F(3/8) = 5/32$ . In this

way, picking a wide range of  $v$  values we will be able to determine the values for the function  $F(r)$ . This approach is used extensively in the real world and the example we are considering has been a particular favorite for study. The data determined from such experiments are shown in Figure 1.3.

A number of conclusions can be drawn from Figure 1.3. For example, there is a range of  $Re$  values where  $G$  is approximately constant. Specifically, if  $10^3 < Re < 10^5$  then  $G \approx 0.7$ . This is the reason why in the fluid dynamics literature you will occasionally see the statement that the drag coefficient  $C_D = \frac{2}{\pi}G$  for a sphere has a constant value of approximately 0.44. For other  $Re$  values, however,  $G$  is not constant. Of particular interest, is the dependence of  $G$  for small values of  $Re$ . This corresponds to velocities  $v$  that are very small, what is known as Stokes flow. The data in Figure 1.3 show that  $G$  decreases linearly with  $Re$  in this region. Given that this is a log-log plot, then this means that  $\log(G) = a - b \log(Re)$ , or equivalently,  $G = \alpha/Re^b$  where  $\alpha = 10^a$ . Curve fitting this function to the data in Figure 1.3 it is found that  $\alpha \approx 17.6$  and  $\beta \approx 1.07$ . These are close to the exact values of  $\alpha = 6\pi$  and  $\beta = 1$ , which are obtained by solving the equations of motion for Stokes flow. Inserting these values into (1.19), the conclusion is that the drag on the sphere for small values of the Reynolds number is

$$D_F \approx 6\pi\mu Rv. \quad (1.22)$$

This is known as Stokes formula for the drag on a sphere, and we will have use for it in Chapter 4 when studying diffusion.

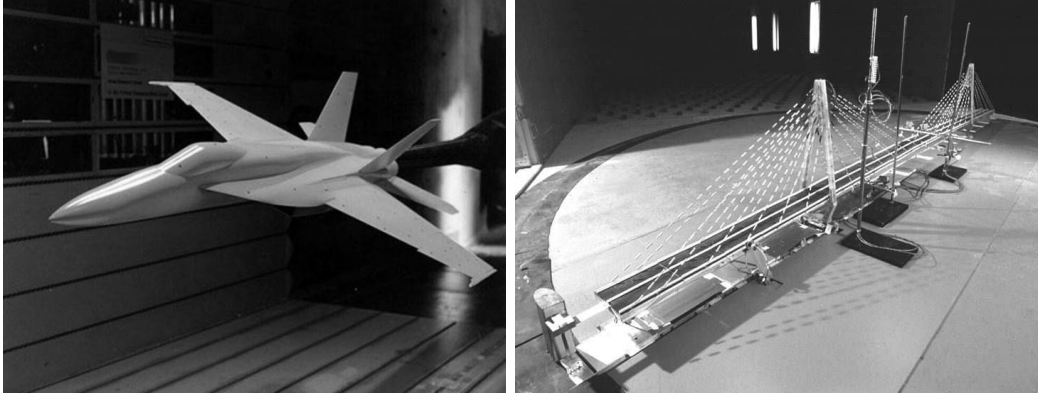
## Scale Models

Why all the work to find  $F$ ? Well, knowing this function allows for the use of scale model testing. To explain, suppose it is required to determine the drag on a sphere with radius  $R_f$  for a given velocity  $v_f$  when the fluid has density  $\rho_f$  and viscosity  $\mu_f$ . Based on (1.17) we have  $D_F = \rho_f R_f^2 v_f^2 F(\Pi_f)$ , where

$$\Pi_f = \frac{\mu_f}{R_f v_f \rho_f}. \quad (1.23)$$

Consequently, we can determine  $D_F$  if we know the value of  $F$  at  $\Pi_f$ . Also, suppose that this cannot be measured directly as  $R_f$  is large and our experimental equipment can only handle small spheres. We can still measure  $F(\Pi_f)$  using a small value of  $R$  if we change one or more of the parameters in such a way that the value of  $\Pi_f$  does not change. If  $R_m, \mu_m, \rho_m$  and  $v_m$  are the values used in the experiment then we want to select them so that

$$\frac{\mu_m}{R_m v_m \rho_m} = \frac{\mu_f}{R_f v_f \rho_f}, \quad (1.24)$$



**Figure 1.4** Scale model testing. Dimensional analysis is used in the development of scale models used in wind tunnels. On the left there is a flight test of an F-18 model in NASA’s 11 ft transonic wind tunnel (NASA [2008]), and on the right a wind tunnel test of a 1:160 scale model of the Owensboro Bridge (Hsu [2009]).

or equivalently

$$v_m = \frac{\mu_m R_f \rho_f}{\mu_f R_m \rho_m} v_f. \quad (1.25)$$

This equation relates the values for the full-scale ball (subscript  $f$ ) to those for the model used in the experiment (subscript  $m$ ). As an example, suppose we are interested in the drag on a very large sphere, say  $R_f = 100\text{ m}$ , but our equipment can only handle smaller values, say  $R_m = 2\text{ m}$ . If the fluid for the two cases is the same, so  $\rho_m = \rho_f$  and  $\mu_m = \mu_f$ , then according to (1.25), in our experiment we should take  $v_m = 50v_f$ . If the experimental apparatus is unable to generate velocities 50 times the value of  $v_f$  then it would be necessary to use a different fluid to reduce this multiplicative factor.

The result in the above example is the basis of scale model testing used in wind tunnels (see Figure 1.4). Usually these tests involve more than just keeping one dimensionless product constant as we did in (1.24). Moreover, it is evident in Figure 1.4 that the models look like the originals, they are just smaller. This is the basis of geometric similarity, where the lengths of the model are all a fraction of the original. For example, the bridge in Figure 1.4 is a  $\frac{1}{20}$ th scale model of the Owensboro Bridge. Other scalings are sometimes used and the most common are kinematic similarity, where velocities are scaled, and dynamic similarity, where forces are scaled.

## Endnotes

One question that has not been considered so far is, how do you know to assume that the drag force depends on the radius, velocity, density, and dynamic viscosity? The assumption comes from knowing the laws of fluid dynamics, and identifying the principal terms that contribute to the drag.



For the most part, in this chapter the assumptions will be stated explicitly, as they were in this example. Later in the text, after the basic physical laws are developed, it will be possible to construct the assumptions directly. However, one important observation can be made, and that is the parameters used in the assumption should be independent. For example, even though the drag on a sphere likely depends on the surface area and volume of the sphere it is not necessary to include them in the list. The reason is that it is already assumed that  $D_F$  depends on the radius  $R$  and both the surface area and volume are determined using  $R$ .

The problem of determining the drag on a sphere is one of the oldest in fluid dynamics. Given that the subject is well over 150 years old, you would think that whatever useful information can be derived from this particular problem was figured out long ago. Well, apparently not, as research papers still appear regularly on this topic. A number of them come from the sports industry, where there is interest in the drag on soccer balls (Asai et al. [2007]), golf balls (Smits and Ogg [2004]), tennis balls (Goodwill et al. [2004]), as well as nonspherical-shaped balls (Mehta [1985]). Others have worked on how to improve the data in Figure 1.3, and an example is the use of a magnetic suspension system to hold the sphere (Sawada and Kunimasu [2004]). A more novel idea is to drop different types of spheres down a deep mine shaft, and then use the splash time as a means to determine the drag coefficient (Maroto et al. [2005]). The point here is that even the most studied problems in science and engineering still have interesting questions that remain unanswered.

### 1.2.3 *Toppling Dominoes*

Domino toppling refers to the art of setting up dominoes, and then knocking them down. The current world record for this is 4,000,000 plus dominoes for a team, and 300,000 plus for an individual. One of the more interesting aspects of this activity is that as the dominoes fall it appears as if a wave is propagating along the line of dominoes. The objective of this example is to examine what dimensional analysis might be able to tell us about the velocity of this wave. A schematic of the situation is shown in Figure 1.5. The assumption is that the velocity  $v$  depends on the spacing  $d$ , height  $h$ , thickness  $t$ , and the gravitational acceleration constant  $g$ . Therefore, the modeling assumption is  $v = f(d, h, t, g)$  and we want to use dimensional reduction to find a simplified version of this expression. As usual, the first step is to find numbers  $a$ ,  $b$ ,  $c$ ,  $e$  so that

$$[v] = [d^a h^b t^c g^e].$$

Expressing these using fundamental dimensions yields

$$\begin{aligned} LT^{-1} &= L^a L^b L^c (L/T^2)^e \\ &= L^{a+b+c+e} T^{-2e}. \end{aligned}$$

Equating the respective terms we obtain

$$\begin{aligned} L : \quad & a + b + c + e = 1, \\ T : \quad & -2e = -1. \end{aligned}$$

Solving these two equations gives us that  $e = \frac{1}{2}$  and  $b = \frac{1}{2} - a - c$ . With this we have that

$$\begin{aligned} v &= \alpha d^a h^{1/2-a-c} t^c g^{1/2} \\ &= \alpha \sqrt{hg} \left( \frac{d}{h} \right)^a \left( \frac{t}{h} \right)^c \\ &= \alpha \sqrt{hg} \Pi_1^a \Pi_2^c, \end{aligned} \tag{1.26}$$

where  $\alpha$  is an arbitrary number, and the two dimensionless products are

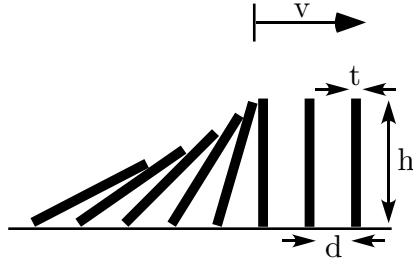
$$\begin{aligned} \Pi_1 &= \frac{d}{h}, \\ \Pi_2 &= \frac{t}{h}. \end{aligned}$$

The expression in (1.26) is the general product solution. Therefore, the general solution for how the velocity depends on the given parameters is

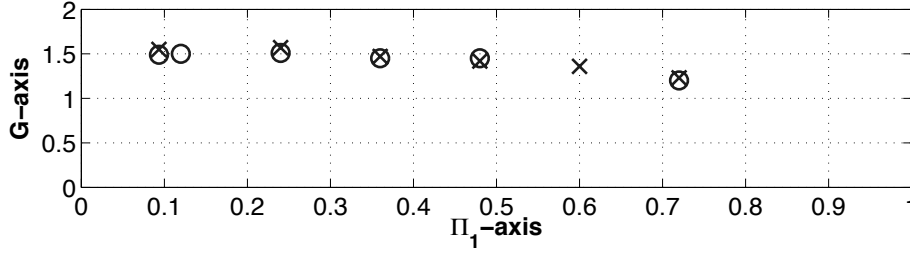
$$v = \sqrt{hg} F(\Pi_1, \Pi_2), \tag{1.27}$$

where  $F$  is an arbitrary function of the two dimensionless products. The proof of how (1.27) follows from (1.26) is very similar to the method used to derive (1.17) from (1.15).

Dimensional analysis has been able to reduce the original assumption involving a function of four-dimensional parameters down to one involving two dimensionless products. This example is also informative as it demonstrates how to obtain the general solution when more than one dimensionless product is involved. The question remains, however, if this really applies to toppling



**Figure 1.5** Schematic of toppling dominoes, creating a wave that propagates with velocity  $v$ .



**Figure 1.6** Data for two different types of toppling dominoes (Stronge and Shu [1988]). In these experiments,  $t = 0.12h$ , so the thin domino approximation is appropriate.

dominoes. It does, but in using this formula it is usually assumed the dominoes are very thin, or more specifically that  $t \ll h$ . This means that it is possible to assume  $\Pi_2 = 0$ , and (1.27) simplifies to

$$v = \sqrt{hg} G(\Pi_1), \quad (1.28)$$

where  $G$  is an arbitrary function. Some effort has been made to measure  $G$ , and the measurements for two different types of dominoes are given in Figure 1.6. Although the data show that  $G$  decreases with  $\Pi_1$ , it is approximately constant over the range of  $\Pi_1$  values used in the experiments. Therefore, as an approximation we conclude that the speed at which dominoes topple is  $v \approx 1.5\sqrt{hg}$ . A typical domino has  $h = 5$  cm, which results in a velocity of  $v \approx 1$  m/s. To obtain a more explicit formula for  $G$ , however, requires the solution of a challenging mathematical problem, and an expanded discussion of this can be found in Efthimiou and Johnson [2007].

### 1.2.4 Endnotes

Based on the previous examples, the benefits of using dimensional reduction are apparent. However, a word of caution is needed here as the method gives the impression that it is possible to derive useful information without getting involved with the laws of physics or potentially difficult mathematical problems. One consequence of this is that the method is used to comment on situations and phenomena that are simply inappropriate (e.g., to study psychoacoustic behavior). The method relies heavily on knowing the fundamental laws for the problem under study, and without this whatever conclusions made using dimensional reduction are limited. For example, we earlier considered the drag on a sphere and in the formulation of the problem we assumed that the drag depends on the dynamic viscosity. Without knowing the equations of motion for fluids it would not have been possible to know that this term needed to be included or what units it might have. By not in-

cluding it we would have concluded that  $d = 0$  in (1.14) and instead of (1.17) we would have  $D_F = \alpha \rho R^2 v^2$  where  $\alpha$  is a constant. In Figure 1.3 it does appear that  $D_F$  is approximately independent of  $Re$  when  $10^3 < Re < 10^5$ . However, outside of this interval,  $D_F$  is strongly dependent on  $Re$ , and this means ignoring the viscosity would be a mistake. Another example illustrating the need to know the underlying physical laws arises in the projectile problem when we included the gravitational constant. Again, this term is essential and without some understanding of Newtonian mechanics it would be missed completely. The point here is that dimensional reduction can be a very effective method for simplifying complex relationships, but it is based heavily on knowing what the underlying laws are that govern the systems being studied.

### 1.3 Theoretical Foundation

The theoretical foundation for dimensional reduction is contained in the Buckingham Pi Theorem. To derive this result assume we have a physical quantity  $q$  that depends on physical parameters or variables  $p_1, p_2, \dots, p_n$ . In this context, the word physical means that the quantity is measurable. Each can be expressed in fundamental dimensions and we will assume that the  $L, T, M$  system is sufficient for this task. In this case we can write

$$[q] = L^{\ell_0} T^{t_0} M^{m_0}, \quad (1.29)$$

and

$$[p_i] = L^{\ell_i} T^{t_i} M^{m_i}. \quad (1.30)$$

Our modeling assumption is that  $q = f(p_1, p_2, \dots, p_n)$ . To dimensionally reduce this expression we will determine if there are numbers  $a_1, a_2, \dots, a_n$  so that

$$[q] = [p_1^{a_1} p_2^{a_2} \cdots p_n^{a_n}]. \quad (1.31)$$

Introducing (1.29) and (1.30) into the above expression, and then equating exponents, we obtain the equations

$$\begin{aligned} L : \quad & \ell_1 a_1 + \ell_2 a_2 + \cdots + \ell_n a_n = \ell_0, \\ T : \quad & t_1 a_1 + t_2 a_2 + \cdots + t_n a_n = t_0, \\ M : \quad & m_1 a_1 + m_2 a_2 + \cdots + m_n a_n = m_0. \end{aligned}$$

This can be expressed in matrix form as

$$\mathbf{A}\mathbf{a} = \mathbf{b}, \quad (1.32)$$

where

$$\mathbf{A} = \begin{pmatrix} \ell_1 & \ell_1 & \cdots & \ell_n \\ t_1 & t_2 & \cdots & t_n \\ m_1 & m_2 & \cdots & m_n \end{pmatrix}, \quad (1.33)$$

$$\mathbf{a} = \begin{pmatrix} a_1 \\ a_2 \\ \vdots \\ a_n \end{pmatrix}, \quad \mathbf{b} = \begin{pmatrix} \ell_0 \\ t_0 \\ m_0 \end{pmatrix}. \quad (1.34)$$

The matrix  $\mathbf{A}$  is known as the dimension matrix. As expressed in (1.33) it is  $3 \times n$  but if we were to have used  $L, T, M, \theta$  as the fundamental system then it would be  $4 \times n$ . In other words, the number of rows in the dimension matrix equals the number of fundamental units needed, and the number of columns equals the number of parameters that  $q$  is assumed to depend on.

With (1.32) we have transformed the dimensional reduction question into a linear algebra problem. To determine the consequences of this we first consider the situation that (1.32) has no solution. In this case the assumption that  $q$  depends on  $p_1, p_2, \dots, p_n$  is incomplete and additional parameters are needed. This situation motivates the following definition.

**Definition 1.1.** The set  $p_1, p_2, \dots, p_n$  is dimensionally incomplete for  $q$  if it is not possible to combine the  $p_i$ 's to produce a quantity with the same dimension as  $q$ . If it is possible, the set is dimensionally complete for  $q$ .

From this point on we will assume the  $p_i$ 's are complete and there is at least one solution of (1.32). To write down the general solution we consider the associated homogeneous equation, namely  $\mathbf{A}\mathbf{a} = \mathbf{0}$ . The set of solutions of this equation form a subspace  $K(\mathbf{A})$ , known as the kernel of  $\mathbf{A}$ . Letting  $k$  be the dimension of this subspace then the general solution of  $\mathbf{A}\mathbf{a} = \mathbf{0}$  can be written as  $\mathbf{a} = \gamma_1 \mathbf{a}_1 + \gamma_2 \mathbf{a}_2 + \cdots + \gamma_k \mathbf{a}_k$ , where  $\mathbf{a}_1, \mathbf{a}_2, \dots, \mathbf{a}_k$  is a basis for  $K(\mathbf{A})$  and  $\gamma_1, \gamma_2, \dots, \gamma_k$  are arbitrary. It is understood here that if  $k = 0$  then  $\mathbf{a} = \mathbf{0}$ . With this, the general solution of (1.32) can be written as

$$\mathbf{a} = \mathbf{a}_p + \gamma_1 \mathbf{a}_1 + \gamma_2 \mathbf{a}_2 + \cdots + \gamma_k \mathbf{a}_k, \quad (1.35)$$

where  $\mathbf{a}_p$  is any vector that satisfies (1.32) and  $\gamma_1, \gamma_2, \dots, \gamma_k$  are arbitrary numbers.

### Example: Drag on a Sphere

To connect the above discussion with what we did earlier consider the drag on a sphere example. Writing (1.13) in matrix form we obtain

$$\begin{pmatrix} 1 & 1 & -3 & -1 \\ 0 & -1 & 0 & -1 \\ 0 & 0 & 1 & 1 \end{pmatrix} \begin{pmatrix} a \\ b \\ c \\ d \end{pmatrix} = \begin{pmatrix} 1 \\ -2 \\ 1 \end{pmatrix}.$$

This is the matrix equation (1.32) for this particular example. Putting this in augmented form, and row reducing, yields the following

$$\left(\begin{array}{cccc|c} 1 & 1 & -3 & -1 & 1 \\ 0 & -1 & 0 & -1 & -2 \\ 0 & 0 & 1 & 1 & 1 \end{array}\right) \rightarrow \left(\begin{array}{cccc|c} 1 & 0 & 0 & 1 & 2 \\ 0 & 1 & 0 & 1 & 2 \\ 0 & 0 & 1 & 1 & 1 \end{array}\right).$$

From this we conclude that  $a = 2 - d$ ,  $b = 2 - d$ , and  $c = 1 - d$ . To be consistent with the notation in (1.35), set  $d = \gamma$ , so the solution is

$$\begin{pmatrix} a \\ b \\ c \\ d \end{pmatrix} = \begin{pmatrix} 2 \\ 2 \\ 1 \\ 0 \end{pmatrix} + \gamma \begin{pmatrix} -1 \\ -1 \\ -1 \\ 1 \end{pmatrix},$$

where  $\gamma$  is arbitrary. Comparing this with (1.35) we have that  $k = 1$ ,

$$\mathbf{a}_p = \begin{pmatrix} 2 \\ 2 \\ 1 \\ 0 \end{pmatrix}, \quad \text{and} \quad \mathbf{a}_1 = \begin{pmatrix} -1 \\ -1 \\ -1 \\ 1 \end{pmatrix}. \quad \blacksquare$$

It is now time to take our linear algebra conclusions and apply them to the dimensional reduction problem. Just as the appearance of  $d$  in (1.14) translated into the appearance of a dimensionless product in the general solution given in (1.17), each of the  $\gamma_i$ 's in (1.35) gives rise to a dimensionless product in the general solution for the problem we are currently studying. To be specific, writing the  $i$ th basis vector  $\mathbf{a}_i$  in component form as

$$\mathbf{a}_i = \begin{pmatrix} \alpha \\ \beta \\ \vdots \\ \gamma \end{pmatrix}, \tag{1.36}$$

then the corresponding dimensionless product is

$$\Pi_i = p_1^\alpha p_2^\beta \cdots p_n^\gamma. \tag{1.37}$$

Moreover, because the  $\mathbf{a}_i$ 's are independent vectors, the dimensionless products  $\Pi_1, \Pi_2, \dots, \Pi_k$  are independent.

As for the particular solution  $\mathbf{a}_p$  in (1.35), assuming it has components

$$\mathbf{a}_p = \begin{pmatrix} a \\ b \\ \vdots \\ c \end{pmatrix}, \quad (1.38)$$

then the quantity

$$Q = p_1^a p_2^b \cdots p_n^c \quad (1.39)$$

has the same dimensions as  $q$ .

Based on the conclusions of the previous two paragraphs, the general product solution is  $q = \alpha Q \Pi_1^{\kappa_1} \Pi_2^{\kappa_2} \cdots \Pi_k^{\kappa_k}$ , where  $\alpha, \kappa_1, \kappa_2, \dots, \kappa_k$  are arbitrary constants. From this we obtain the following theorem.

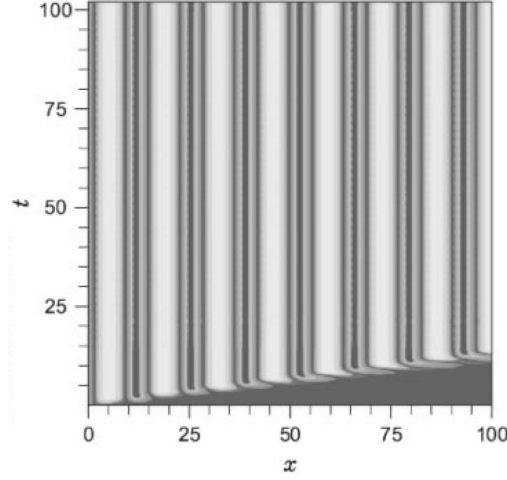
**Theorem 1.1.** *Assuming the formula  $q = f(p_1, p_2, \dots, p_n)$  is dimensionally homogeneous and dimensionally complete, then it is possible to reduce it to one of the form  $q = Q F(\Pi_1, \Pi_2, \dots, \Pi_k)$ , where  $\Pi_1, \Pi_2, \dots, \Pi_k$  are independent dimensionless products of  $p_1, p_2, \dots, p_n$ . The quantity  $Q$  is a dimensional product of  $p_1, p_2, \dots, p_n$  with the same dimensions as  $q$ .*

According to this theorem, the original formula for  $q$  can be reduced from a function of  $n$  variables down to one with  $k$ . The value of  $k$ , which equals the nullity of the dimension matrix, ranges from 0 to  $n - 1$  depending on the given quantities  $p_1, p_2, \dots, p_n$ . In the case that  $k = 0$  the function  $F$  reduces to a constant and the conclusion is that  $q = \alpha Q$ , where  $\alpha$  is an arbitrary number.

The importance of this theorem is that it establishes that the process used to reduce the drag on a sphere and toppling dominoes examples can be applied to much more complex problems. It also provides insight into how the number of dimensionless products is determined. There are still, however, fundamental questions left unanswered. For example, those with a more mathematical bent might still be wondering if this result can really be true no matter how discontinuous the original function  $f$  might be. Others might be wondering if the fundamental units used here, particularly length and time, are really independent. This depth of inquiry, although quite interesting, is beyond the scope of this text. Those wishing to pursue further study of these and related topics should consult Penrose [2007] and Bluman and Anco [2002].

### 1.3.1 Pattern Formation

The mechanism responsible for the colorful patterns on seashells, butterfly wings, zebras, and the like has intrigued scientists for decades. An experiment that has been developed to study pattern formation involves pouring chemicals into one end of a long tube, and then watching what happens as they interact while moving along the tube. This apparatus is called a plug-flow



**Figure 1.7** Spatial pattern created in a plug-flow reactor (Bamforth et al. [2000]). The tube occupies the interval  $0 \leq x \leq 100$ , and starting at  $t = 0$  the chemicals are poured into the left end. As they flow along the tube a striped pattern develops.

reactor and the outcome of one such experiment is shown in Figure 1.7. It was found in these experiments that patterns appear only for certain pouring velocities  $v$ . According to what is known as the Lengyel-Epstein model, this velocity depends on the concentration  $U$  of the chemical used in the experiment, the rate  $k_2$  at which the chemicals interact, the diffusion coefficient  $D$  of the chemicals, and a parameter  $k_3$  that has the dimensions of concentration squared. The model is therefore assuming

$$v = f(U, k_2, D, k_3). \quad (1.40)$$

From Table 1.1 we have that  $[v] = L/T$ ,  $[U] = 1/L^3$ ,  $[D] = L^2/T$ , and  $[k_3] = 1/L^6$ . Also, from the Lengyel-Epstein model one finds that  $[k_2] = L^3/T$ . Using dimensional reduction we require

$$[v] = [U^a k_2^b D^c k_3^d]. \quad (1.41)$$

Expressing these using fundamental dimensions yields

$$\begin{aligned} LT^{-1} &= (L^{-3})^a (L^3 T^{-1})^b (L^2 T^{-1})^c (L^{-6})^d \\ &= L^{-3a+3b+2c-6d} T^{-b-c}. \end{aligned}$$

As before we equate the respective terms and conclude

$$\begin{aligned} L : -3a + 3b + 2c - 6d &= 1 \\ T : -b - c &= -1. \end{aligned}$$

These equations will enable us to express two of the unknowns in terms of the other two. There is no unique way to do this, and one choice yields



$b = -1 + 3a + 6d$  and  $c = 2 - 3a - 6d$ . From this it follows that the general product solution is

$$\begin{aligned} v &= \alpha U^a k_2^{3a+6d-1} D^{2-3a-6d} k_3^d \\ &= \alpha k_2^{-1} D^2 (U k_2^3 D^{-3})^a (k_2^6 D^{-6} k_3)^d. \end{aligned}$$

This can be rewritten as

$$v = \alpha k_2^{-1} D^2 \Pi_1^a \Pi_2^d, \quad (1.42)$$

where

$$\Pi_1 = \frac{U k_2^3}{D^3}, \quad (1.43)$$

and

$$\Pi_2 = \frac{k_2^6 k_3}{D^6}. \quad (1.44)$$

The dimensionless products  $\Pi_1$  and  $\Pi_2$  are independent, and this follows from the method used to derive these expressions. Independence is also evident from the observation that  $\Pi_1$  and  $\Pi_2$  do not involve exactly the same parameters. From this result it follows that the general form of the reduced equation is

$$v = k_2^{-1} D^2 F(\Pi_1, \Pi_2). \quad (1.45)$$

It is of interest to compare (1.45) with the exact formula obtained from solving the differential equations coming from the Lengyel-Epstein model. It is found that

$$v = \sqrt{k_2 D U} G(\beta), \quad (1.46)$$

where  $\beta = k_3/U^2$  and  $G$  is a rather complicated square root function (Bamforth et al. [2000]). This result appears to differ from (1.45). To investigate this, note that  $\beta = \Pi_2/\Pi_1^2$ . Equating (1.45) and (1.46) it follows that

$$\begin{aligned} F(\Pi_1, \Pi_2) &= \frac{k_2^{3/2} U^{1/2}}{D^{3/2}} G(\beta) \\ &= \sqrt{\Pi_1} G(\Pi_2/\Pi_1^2). \end{aligned}$$

Because the right-hand side is a function of only  $\Pi_1$  and  $\Pi_2$  then (1.45) does indeed reduce to the exact result (1.46). Dimensional reduction has therefore successfully reduced the original unknown function of four variables in (1.40) down to one with only two variables. However, the procedure is not able to reduce the function down to one dimensionless variable, as given in (1.46). In this problem that level of reduction requires information only available from the differential equations, something that dimensional arguments are not able to discern.

## 1.4 Similarity Variables

Dimensions can be used not just to reduce formulas, they can be also used to simplify complex mathematical problems. The degree of simplification depends on the parameters, and variables, in the problem. One of the more well-known examples is the problem of finding the density  $u(x, t)$  of a chemical over the interval  $0 < x < \infty$ . In this case the density satisfies the diffusion equation

$$D \frac{\partial^2 u}{\partial x^2} = \frac{\partial u}{\partial t}, \quad (1.47)$$

where the boundary conditions are

$$u|_{x=0} = u_0, \quad u|_{x \rightarrow \infty} = 0, \quad (1.48)$$

and the initial condition is

$$u|_{t=0} = 0. \quad (1.49)$$

The constant  $D$  is called the diffusion coefficient, and its dimensions can be determined from the terms in the differential equation. In particular, the dimensions of the left and right sides of (1.47) must be the same, and this means  $[Du_{xx}] = [u_t]$ . Because  $[u] = M/L^3$  then  $[u_{xx}] = [u]/L^2 = M/L^5$  and  $[u_t] = [u]/T = M/(TL^3)$ . From this we have  $[D]M/L^5 = M/(TL^3)$ , and therefore  $[D] = L^2/T$ . In a similar manner, in boundary condition (1.48),  $[u_0] = [u] = M/L^3$ . As a final comment, the physical assumptions underlying the derivation of (1.47) are the subject of Chapter 4. In fact, the solution we are about to derive is needed in Section 4.5.2 to solve the diffusion equation.

## Dimensional Reduction

The conventional method for solving the diffusion equation on a semi-infinite spatial interval is to use an integral transform, and this will be considered in Chapter 4. It is also possible to find  $u$  using dimensional reduction. The approach is based on the observation that the only dimensional variables, and parameters, appearing in the problem are  $u$ ,  $u_0$ ,  $D$ ,  $x$ , and  $t$ . In other words, it must be true that  $u = f(x, t, D, u_0)$ . With this we have the framework for dimensional reduction, and the question is whether we can find numbers  $a, b, c, d$  so that

$$[u] = [x^a t^b D^c (u_0)^d]. \quad (1.50)$$

Using fundamental dimensions yields

$$\begin{aligned} ML^{-3} &= L^a T^b (L^2/T)^c (M/L^3)^d \\ &= L^{a+2c-3d} T^{b-c} M^d, \end{aligned}$$

and then equating the respective terms gives us

$$\begin{aligned}
L : a + 2c - 3d &= -3, \\
T : b - c &= 0, \\
M : d &= 1.
\end{aligned}
\tag{1.51}$$

The solution of the above system can be written as  $d = 1$  and  $b = c = -a/2$ . Given the assumption in (1.50), we conclude that the general product solution is

$$u = \alpha u_0 \left( \frac{x}{\sqrt{Dt}} \right)^a.$$

The general solution therefore has the form

$$u = u_0 F(\eta), \tag{1.52}$$

where

$$\eta = \frac{x}{\sqrt{Dt}}. \tag{1.53}$$

In this case,  $\eta$  is called a *similarity variable* as it is a dimensionless product that involves the independent variables in the problem.

When working out the drag on a sphere example, we discussed how it is possible to derive different representations of the solution. For the current example, when solving (1.51), instead of writing  $b = c = -a/2$ , we could just as well state that  $a = -2b$  and  $c = b$ . In this case (1.52) is replaced with  $u = u_0 G(\xi)$  where  $\xi = Dt/x^2$ . Although the two representations are equivalent, in the sense that one can be transformed into the other, it does make a difference which one is used when deriving a similarity solution. The reason is that we will be differentiating the solution, and (1.52) leads to much simpler formulas than the other representation. The rule of thumb here is that you want  $x$  in the numerator of the similarity variable. If you would like a hands on example of why this is true, try working out the steps below using the representation  $u = u_0 G(\xi)$  instead of (1.52).

## Similarity Solution

Up to this point we have been using a routine dimensional reduction argument. Our result, given in (1.52), is interesting as it states that the solution has a very specific dependence on the independent variables  $x$  and  $t$ . Namely,  $u$  can be written as a function of a single intermediate variable  $\eta$ . To determine  $F$  we substitute (1.52) back into the problem and find what equation  $F$  satisfies. With this in mind note, using the chain rule,

$$\begin{aligned}
\frac{\partial u}{\partial t} &= u_0 F'(\eta) \frac{\partial \eta}{\partial t} \\
&= u_0 F'(\eta) \left( -\frac{x}{2D^{1/2}t^{3/2}} \right) \\
&= -u_0 F'(\eta) \frac{\eta}{2t}.
\end{aligned}$$

In a similar manner one can show that

$$\frac{\partial^2 u}{\partial x^2} = u_0 F''(\eta) \frac{1}{Dt}.$$

Substituting these into (1.47) yields

$$F'' = -\frac{1}{2}\eta F', \quad \text{for } 0 < \eta < \infty. \quad (1.54)$$

We must also transform the boundary and initial conditions. The boundary condition at  $x = 0$  takes the form

$$F(0) = 1, \quad (1.55)$$

while the condition as  $x \rightarrow \infty$  and the one at  $t = 0$  both translate into

$$F(\infty) = 0. \quad (1.56)$$

With this we have transformed a problem involving a partial differential equation (PDE) into one with an ordinary differential equation (ODE). As required, the resulting problem for  $F$  is only in terms of  $\eta$ . All of the original dimensional quantities, including the independent variables  $x$  and  $t$ , do not appear anywhere in the problem. This applies not just to the differential equation, but also to the boundary and initial conditions.

The problem for  $F$  is simpler than the original diffusion problem and, by itself, makes the use of dimensional analysis worthwhile. In this particular problem it is so simple that it is possible to solve for  $F$ . This can be done by letting  $G = F'$ , so the equation takes the form  $G' = -\frac{1}{2}\eta G$ . The general solution of this is  $G = \alpha \exp(-\eta^2/4)$ . Because  $F' = G$ , we conclude that the general solution is

$$F(\eta) = \beta + \alpha \int_0^\eta e^{-s^2/4} ds. \quad (1.57)$$

From (1.55) we have that  $\beta = 1$  and from (1.56) we get

$$1 + \alpha \int_0^\infty e^{-s^2/4} ds = 0. \quad (1.58)$$

Given that  $\int_0^\infty e^{-s^2/4} ds = \sqrt{\pi}$ , then

$$F(\eta) = 1 - \frac{1}{\sqrt{\pi}} \int_0^\eta e^{-s^2/4} ds. \quad (1.59)$$

Expressions like this arise so often that they have given rise to a special function known as the complementary error function  $\operatorname{erfc}(\eta)$ . This is defined as

$$\operatorname{erfc}(\eta) = 1 - \frac{2}{\sqrt{\pi}} \int_0^\eta e^{-r^2} dr. \quad (1.60)$$

Therefore, we have found that the solution of the diffusion problem is

$$u(x, t) = u_0 \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right). \quad (1.61)$$

As the above example demonstrates, using similarity variables and dimensional analysis provides a powerful tool for solving PDEs. It is, for example, one of the very few methods known that can be used to solve nonlinear problems. Its limitation is that the problem must have a specific form to work. We were able to solve the above diffusion problem because dimensional analysis reduced the form of the solution down to a function of one variable. This does not always happen and in such cases the method provides no insight into how the problem can be solved. As an example, if the spatial interval in the above diffusion problem is changed to one that is finite, so  $0 < x < \ell$ , then dimensional analysis will show that there are two independent similarity variables. This represents no improvement as we already know it is a function of two independent variables, so a reduction is not possible. Even with these limitations, however, similarity variables and their use in solving differential equations is a thriving area and a good introduction of the material can be found in Bluman and Cole [1974].

## 1.5 Nondimensionalization and Scaling

Another use we will have for dimensional analysis is to transform a problem into dimensionless form. The reason for this is that the approximation methods that are used to reduce difficult problems are based on comparisons. For example, in the projectile problem we simplified the differential equation by assuming that  $x$  was small compared to  $R$ . In contrast there are problems where the variable of interest is large, or it is slow or that it is fast compared to some other term in the problem. Whatever the comparison, it is important to know how all of the terms in the problem compare and for this we need the concept of scaling.

### 1.5.1 Projectile Problem

The reduction of the projectile equation (1.1) was based on the assumption that  $x$  is not very large, and so  $x + R$  could be replaced with just  $R$ . We will routinely use arguments like this to find an approximate solution and it is therefore essential we take more care in making such reductions. The way this is done is by first scaling the variables in the problem using characteristic values. The best way to explain what this means is to work out an example and the projectile problem is an excellent place to start.

#### Change Variables

The first step in nondimensionalizing a problem is to introduce a change of variables, which for the projectile problem will have the form

$$\begin{aligned} t &= t_c s, \\ x &= x_c u. \end{aligned}$$

In the above formula,  $x_c$  is a constant and it is a characteristic value of the variable  $x$ . It is going to be determined using the physical parameters in the problem, which for the projectile problem are  $g$ ,  $R$ , and  $v_0$ . In a similar manner,  $t_c$  is a constant that has the dimensions of time and it represents a characteristic value of the variable  $t$ . In some problems it will be clear at the beginning how to select  $x_c$  and  $t_c$ . However, it is assumed here that we have no clue at the start what to choose and will not select them until the problem is studied a bit more. All we know at the moment is that whatever the choice, the new variables  $u, s$  are dimensionless. To make the change of variables note that from the chain rule

$$\begin{aligned} \frac{d}{dt} &= \frac{ds}{dt} \frac{d}{ds} \\ &= \frac{1}{t_c} \frac{d}{ds}, \end{aligned} \tag{1.62}$$

and

$$\frac{d^2}{dt^2} = \frac{d}{dt} \left( \frac{d}{dt} \right) = \frac{1}{t_c^2} \frac{d^2}{ds^2}. \tag{1.63}$$

With this the projectile equation (1.1) takes the form

$$\frac{1}{t_c^2} \frac{d^2}{ds^2} (x_c u) = - \frac{gR^2}{(R + x_c u)^2}. \tag{1.64}$$

The method requires us to collect the parameters into dimensionless groups. There is no unique way to do this, and this can cause confusion when first

learning the procedure as there is no fixed method or answer. For example, to nondimensionalize the denominator in (1.64) one can factor it as either  $R(1 + x_c u/R)$  or as  $x_c(R/x_c + u)$ . The first has the benefit of enabling us to cancel the  $R$  in the numerator. Making this choice yields

$$\frac{x_c}{gt_c^2} \frac{d^2 u}{ds^2} = -\frac{1}{(1 + x_c u/R)^2}, \quad (1.65)$$

where the initial conditions (1.2), (1.3) are

$$u(0) = 0, \quad (1.66)$$

$$\frac{du}{ds}(0) = \frac{t_c}{x_c} v_0. \quad (1.67)$$

### Find the Dimensionless Groups

Our change of variables has resulted in three dimensionless groups appearing in the transformed problem. They are

$$\Pi_1 = \frac{x_c}{gt_c^2}, \quad (1.68)$$

$$\Pi_2 = \frac{x_c}{R}, \quad (1.69)$$

$$\Pi_3 = \frac{t_c v_0}{x_c}. \quad (1.70)$$

There are a few important points that need to be made here. First, the  $\Pi$ 's do not involve the variables  $u, s$  and only depend on the parameters in the problem. Second, they are dimensionless and to accomplish this it was necessary to manipulate the projectile problem so the parameters end up grouped together to form dimensionless ratios. The third, and last, point is that the above three dimensionless groups are independent in the sense that it is not possible to write any one of them in terms of the other two. For example,  $\Pi_1$  is the only one that contains the parameter  $g$  while  $\Pi_2$  is the only one containing  $R$ . It is understood that in making the statement that the three groups are independent that  $x_c$  and  $t_c$  can be selected, if desired, independently of any of the parameters in the problem.

Before deciding on how to select  $x_c$  and  $t_c$ , it is informative to look a little closer at the above dimensionless groups. We begin with  $\Pi_2$ . In physical terms it is a measure of a typical, or characteristic, height of the projectile compared to the radius of the Earth. In comparison,  $\Pi_3$  is a measure of a typical, or characteristic, velocity  $x_c/t_c$  compared to the velocity the projectile starts with. Finally, the parameter group  $\Pi_1$  measures a typical, or characteristic, acceleration  $x_c/t_c^2$  in comparison to the acceleration due to

gravity in a uniform field. These observations can be helpful when deciding on how to nondimensionalize a problem as will be shown next.

### Use Dimensionless Groups to Determine Scaling

It is now time to actually decide on what to take for  $x_c$  and  $t_c$ . There are whole papers written on what to consider as you select these parameters, but we will take a somewhat simpler path. For our problem we have two parameters to determine, and we will do this by setting two of the above dimensionless groups equal to one. What we need to do is decide on which two to pick, and we will utilize what might be called rules of thumb.

*Rule of Thumb 1:* Pick the  $\Pi$ 's that appear in the initial and/or boundary conditions.

We only have initial conditions in our problem, and the only dimensionless group involved with them is  $\Pi_3$ . So we set  $\Pi_3 = 1$  and conclude

$$x_c = v_0 t_c. \quad (1.71)$$

*Rule of Thumb 2:* Pick the  $\Pi$ 's that appear in the reduced problem.

To use this rule it is first necessary to explain what the reduced problem is. This comes from the earlier assumption that the object does not get very high in comparison to the radius of the Earth, in other words,  $\Pi_2$  is small. The reduced problem is the one obtained in the extreme limit of  $\Pi_2 \rightarrow 0$ . Taking this limit in (1.65)-(1.67), and using (1.71), the reduced problem is

$$\Pi_1 \frac{d^2 u}{ds^2} = -1,$$

where

$$u(0) = 0, \quad \text{and} \quad \frac{du}{ds}(0) = 1.$$

According to the stated rule of thumb, we set  $\Pi_1 = 1$ , and so

$$x_c = v_0^2/g. \quad (1.72)$$

This choice for  $x_c$  seems reasonable based on our earlier conclusion that the maximum height for the uniform field case is  $v_0^2/(2g)$ .

Combining (1.71) and (1.72), we have that  $x_c = v_0^2/g$  and  $t_c = v_0/g$ . With this scaling then (1.65) - (1.67) take the form

$$\frac{d^2 u}{ds^2} = -\frac{1}{(1 + \epsilon u)^2}, \quad (1.73)$$



where

$$u(0) = 0, \quad (1.74)$$

$$\frac{du}{ds}(0) = 1. \quad (1.75)$$

The dimensionless parameter appearing in the above equation is

$$\epsilon = \frac{v_0^2}{gR}. \quad (1.76)$$

This parameter will play a critical role in our constructing an accurate approximation of the solution of the projectile problem. This will be done in the next chapter but for the moment recall that since  $R \approx 6.4 \times 10^6$  m and  $g \approx 9.8 \text{ m/s}^2$  then  $\epsilon \approx 1.6 \times 10^{-8} v_0^2$ . Consequently for baseball bats, sling shots, BB-guns, and other everyday projectile-producing situations, where  $v_0$  is not particularly large, the parameter  $\epsilon$  is very small. This observation is central to the subject of the next chapter.

### Changing Your Mind

Before leaving this example it is worth commenting on the nondimensionalization procedure by asking a question. Namely, how bad is it if different choices would have been made for  $x_c$  and  $t_c$ ? For example, suppose for some reason one decides to take  $\Pi_2 = 1$  and  $\Pi_3 = 1$ . The resulting projectile problem is

$$\epsilon \frac{d^2 u}{ds^2} = -\frac{1}{(1+u)^2}, \quad (1.77)$$

where  $u(0) = 0$ ,  $\frac{du}{ds}(0) = 1$ , and  $\epsilon$  is given in (1.76). No approximation has been made here and therefore this problem is mathematically equivalent to the one given in (1.73)-(1.75). Based on this, the answer to the question would be that using this other scaling is not so bad. However, the issue is amenability and what properties of the solution one is interested in. To explain, earlier we considered how the solution behaves if  $v_0$  is not very large. With the scaling that produced (1.77), small  $v_0$  translates into looking at what happens when  $\epsilon$  is near zero. Unfortunately, the limit of  $\epsilon \rightarrow 0$  results in the loss of the highest derivative in the differential equation and (1.77) reduces to  $0 = -1$ . How to handle such singular limits will be addressed in the next chapter but it requires more work than is necessary for this problem. In comparison, letting  $\epsilon$  approach zero in (1.73) causes no such complications and for this reason it is more amenable to the study of the small  $v_0$  limit. The point here is that if there are particular limits, or conditions, on the parameters that it is worth taking them into account when constructing the scaling.

### 1.5.2 Weakly Nonlinear Diffusion

To explore possible extensions of the nondimensionalization procedure we consider a well-studied problem involving nonlinear diffusion. The problem consists of finding the concentration  $c(x, t)$  of a chemical over an interval  $0 < x < \ell$ . The concentration satisfies

$$D \frac{\partial^2 c}{\partial x^2} = \frac{\partial c}{\partial t} - \lambda(\gamma - c)c, \quad (1.78)$$

where the boundary conditions are

$$c|_{x=0} = c|_{x=\ell} = 0, \quad (1.79)$$

and the initial condition is

$$c|_{t=0} = c_0 \sin(5\pi x/\ell). \quad (1.80)$$

The nonlinear diffusion equation (1.78) is known as Fisher's equation, and it arises in the study of the movement of genetic traits in a population. A common simplifying assumption made when studying this equation is that the nonlinearity is weak, which means that the term  $\lambda c^2$  is small in comparison to the others in the differential equation. This assumption will be accounted for in the nondimensionalization.

Before starting the nondimensionalization process we should look at the fundamental dimensions of the variables and parameters in the problem. First,  $c$  is a concentration, which corresponds to the number of molecules per unit volume, and so  $[c] = L^{-3}$ . The units for the diffusion coefficient  $D$  were determined earlier, and it was found that  $[D] = L^2/T$ . As for  $\gamma$ , the  $\gamma - c$  term in the differential equation requires these two quantities to have the same dimensions, and so  $[\gamma] = [c]$ . Similarly, from the differential equation we have  $[\lambda(\gamma - c)c] = [\frac{\partial c}{\partial t}]$ , and from this it follows that  $[\lambda] = L^3 T^{-1}$ . Finally, from the initial condition we have that  $[c_0] = [c]$ . It is important to make an observation related to dimensions, and this will be done by asking a question: is it possible to replace the initial condition (1.80) with  $c|_{t=0} = c_0 \sin(5\pi x)$  or with  $c|_{t=0} = c_0 \sin(x)$ ? The answer in both cases is no, and the reason is that the argument of the sine function must be dimensionless. For exactly the same reason it is not possible to use  $c|_{t=0} = c_0 e^x$ . It is possible, however, to use  $c|_{t=0} = c_0 x$  or  $c|_{t=0} = c_0 x^2$ , although the dimensions of  $c_0$  differ from what we found earlier.

Now, to nondimensionalize the problem we introduce the change of variables

$$x = x_c y, \quad (1.81)$$

$$t = t_c s, \quad (1.82)$$

$$c = c_c u. \quad (1.83)$$

In this context,  $x_c$  has the dimensions of length and is a characteristic value of the variable  $x$ . Similar statements apply to  $t_c$  and  $c_c$ . Using the chain rule as in (1.62) the above differential equation takes the form

$$\frac{Dc_c}{x_c^2} \frac{\partial^2 u}{\partial y^2} = \frac{c_c}{t_c} \frac{\partial u}{\partial s} - \lambda c_c (\gamma - c_c u) u.$$

It is necessary to collect the parameters into dimensionless groups, and so in the above equation we rearrange things a bit to obtain

$$\frac{Dt_c}{x_c^2} \frac{\partial^2 u}{\partial y^2} = \frac{\partial u}{\partial s} - \lambda t_c c_c (\gamma/c_c - u) u. \quad (1.84)$$

In conjunction with this we have the boundary conditions

$$u|_{y=0} = u|_{y=\ell/x_c} = 0, \quad (1.85)$$

and the initial condition is

$$u|_{s=0} = (c_0/c_c) \sin(5\pi x_c y/\ell). \quad (1.86)$$

The resulting dimensionless groups are

$$\Pi_1 = \frac{Dt_c}{x_c^2}, \quad (1.87)$$

$$\Pi_2 = \lambda t_c c_c, \quad (1.88)$$

$$\Pi_3 = \gamma/c_c, \quad (1.89)$$

$$\Pi_4 = \ell/x_c, \quad (1.90)$$

$$\Pi_5 = c_0/c_c. \quad (1.91)$$

It is important to note that the five dimensionless groups given above are independent in the sense that it is not possible to write one of them in terms of the other four. As before this statement is based on our ability to select, if desired, the scaling parameters  $x_c, t_c, c_c$  independently of each other and the other parameters in the problem. Also, in counting the dimensionless groups one might consider adding a sixth. Namely, in the initial condition (1.86) there is  $\Pi_6 = 5\pi x_c/\ell$ . The reason it is not listed above is that it is not independent of the others because  $\Pi_6 = 5\pi/\Pi_4$ . The  $5\pi$  is a number and does not play a role in determining dimensional independence.

We have three scaling parameters to specify, namely  $x_c, t_c, c_c$ . Using Rule of Thumb 1, the  $\Pi$ 's that appear in the boundary and initial conditions are

set equal to one. In other words, we set  $\Pi_4 = 1$  and  $\Pi_5 = 1$ , from which it follows that  $x_c = \ell$  and  $c_c = c_0$ .

To use Rule of Thumb 2, we need to investigate what it means to say that the nonlinearity is weak. The equation (1.84) is nonlinear due to the term  $\lambda t_c c_c u^2$ , and the coefficient  $\lambda t_c c_c$  is the associated strength of the nonlinearity. For a weakly nonlinear problem one is interested in the solution for small values of  $\lambda t_c c_c$ . Taking the extreme limit we set  $\lambda t_c c_c = 0$  in (1.84) to produce the reduced equation. The only group that remains in this limit is  $\Pi_1$ , and for this reason this is the group we select. So, setting  $\Pi_1 = 1$  then we conclude  $t_c = \ell^2/D$ .

The resulting nondimensional diffusion equation is

$$\frac{\partial^2 u}{\partial y^2} = \frac{\partial u}{\partial s} - \epsilon(b - u)u, \quad (1.92)$$

with boundary conditions

$$u(0, s) = u(1, s) = 0, \quad (1.93)$$

and the initial condition

$$u(y, 0) = \sin(5\pi y). \quad (1.94)$$

The dimensionless parameters appearing in the above equation are  $\epsilon = \lambda c_0 \ell^2 / D$  and  $b = \gamma / c_0$ . With this, weak nonlinearity corresponds to assuming that  $\epsilon$  is small.

### 1.5.3 Endnotes

As you might have noticed, the assumption of a weak nonlinearity was used in the projectile problem, although it was stated in more physical terms. In both examples the reduced problem, obtained setting  $\epsilon = 0$ , is linear. It is certainly possible that a physical problem is not weakly nonlinear but involves some other extreme behavior. As an example, in nonlinear diffusion problems you come across situations involving weak diffusion. What this means for (1.84) is that  $D t_c / x_c^2$  has a small value. In the extreme limit that this term is zero then the only group that remains in the reduced problem is  $\Pi_2$ . Setting  $\Pi_2 = 1$  then  $t_c = c_0 / \lambda$ . With this, (1.84) becomes

$$\epsilon \frac{\partial^2 u}{\partial x^2} = \frac{\partial u}{\partial t} + (b - u)u, \quad (1.95)$$

where  $\epsilon = D c_0 / (\lambda \ell^2)$  and  $b = \gamma / c_0$ . With this, weak diffusion corresponds to assuming that  $\epsilon$  is small.

For those keeping track of the rules of thumb used to nondimensionalize a problem we have two. The first we ran across is the rule that the dimensionless groups in the initial and boundary conditions are set to one. The second rule arose when setting the dimensionless groups in the reduced problem to one. Although these can be effective rules, it is certainly possible to find problems where another scaling should be considered, and examples are given in Exercises 1.17 and 3.8. The overall objective in all cases is that the nondimensionalization is based on characteristic values of the variables.

## Exercises

**1.1.** The amount of noise permitted from the large rollers used in road construction was recently limited by changes in the environmental laws. Rather than build multiple full-sized rollers in an attempt to find one that satisfied the new law a manufacturer decided that dimensional analysis could be used. The assumption they made was that the frequency  $f$  of the sound coming off the roller depends on the elastic modulus  $E$  and the density  $\rho$  of the steel used to construct the roller as well as on the length  $\ell$  of the roller.

- (a) Find a dimensionally reduced form for  $f$ .
- (b) In building a scale model for testing the manufacturer selected the parameters so that

$$\frac{f_m}{f_f} = \frac{\ell_f}{\ell_m} \sqrt{\frac{\rho_f E_m}{\rho_m E_f}},$$

where the subscript  $f$  designates full-sized and the subscript  $m$  designates scale model. Explain why this was done.

**1.2.** For a pendulum that starts from rest, the period  $p$  depends on the length  $\ell$  of the rod, on gravity  $g$ , on the mass  $m$  of the ball, and on the initial angle  $\theta_0$  at which the pendulum is started.

- (a) Use dimensional analysis to determine the functional dependence of  $p$  on these four quantities.
- (b) For the largest pendulum ever built, the rod is 70 ft and the ball weighs 900 lbs. Assuming that  $\theta_0 = \pi/6$  explain how to use a pendulum that fits on your desk to determine the period of this largest pendulum.
- (c) Suppose it is found that  $p$  depends linearly on  $\theta_0$ , with  $p = 0$  if  $\theta_0 = 0$ . What does your result in part (a) reduce to in this case?

**1.3.** The velocity  $v$  at which flow in a pipe will switch from laminar to turbulent depends on the diameter  $d$  of the pipe as well as on the density  $\rho$  and dynamic viscosity  $\mu$  of the fluid.

- (a) Find a dimensionally reduced form for  $v$ .
- (b) Suppose the pipe has diameter  $d = 100$  and for water (where  $\rho = 1$  and  $\mu = 10^{-2}$ ) it is found that  $v = 0.25$ . What is  $v$  for olive oil (where  $\rho = 1$  and  $\mu = 1$ )? The units here are in cgs.

**1.4.** The luminosity of certain giant and supergiant stars varies in a periodic manner. It is hypothesized that the period  $p$  depends upon the star's average radius  $r$ , its mass  $m$ , and the gravitational constant  $G$ .

- (a) Newton's law of gravitation asserts that the attractive force between two bodies is proportional to the product of their masses divided by the square of the distance between them, that is,

$$F = \frac{Gm_1m_2}{d^2},$$

where  $G$  is the gravitational constant. From this determine the (fundamental) dimensions of  $G$ .

- (b) Use dimensional analysis to determine the functional dependence of  $p$  on  $m$ ,  $r$ , and  $G$ .  
 (c) Arthur Eddington used the theory for thermodynamic heat engines to show that

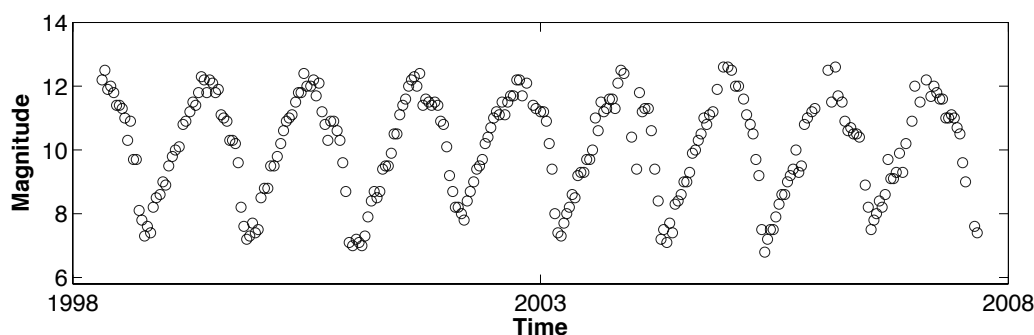
$$p = \sqrt{\frac{3\pi}{2\gamma G\rho}},$$

where  $\rho$  is the average density of the star and  $\gamma$  is the ratio of specific heats for stellar material. How does this differ from your result?

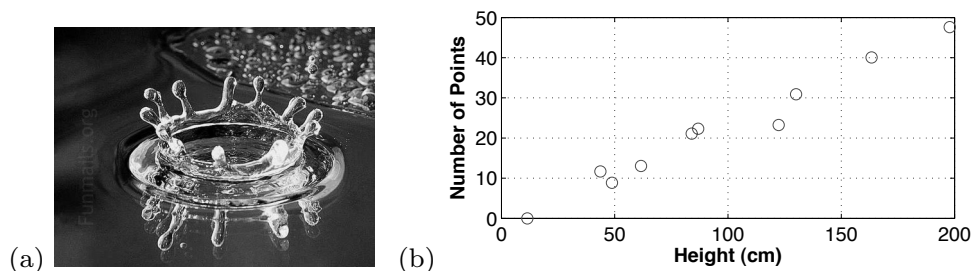
- (d) In Figure 1.8 the data for a pulsating star are given. Explain how you could use data like this to complete the formula you derived in part (b).

**1.5.** When a drop of liquid hits a wetted surface a crown formation appears, as shown in Figure 1.9(a). It has been found that the number of points  $N$  on the crown depends on the speed  $U$  at which the drop hits the surface, the radius  $r$  and density  $\rho$  of the drop, and the surface tension  $\sigma$  of the liquid making up the drop. How  $N$  depends on these quantities has been studied extensively and some of the reasons why are given in Rioboo et al. [2003].

- (a) Use dimensional reduction to determine the functional dependence of  $N$  on  $U$ ,  $r$ ,  $\rho$ , and  $\sigma$ . Express your answer in terms of the Weber number  $W_e = \rho U^2 r / \sigma$ .



**Figure 1.8** Luminosity of a Mira type variable star, 1621+19 U Herculis (AAVSO [2009]).



**Figure 1.9** (a) Formation of a crown when a liquid drop hits a wetted surface. (b) The measured values of the number of points  $N$  (Hobbs and Kezweent [1967]).

- (b) The value of  $N$  has been measured as a function of the initial height  $h$  of the drop and the results are shown in Figure 1.9(b). Express your answer in part (a) in terms of  $h$  by writing  $U$  in terms of  $h$  and  $g$ . Assume the drop starts with zero velocity.
- (c) The data in Figure 1.9(b) show a piecewise linear dependence on  $h$ , specifically,  $N$  can be described as a continuous function made up of two linear segments. Use this, and your result from part (b), to find the unknown function in part (a). In the experiments,  $r = 3.6$  mm,  $\rho = 1.1014$  gm/cm<sup>3</sup>, and  $\sigma = 50.5$  dyn/cm.
- (d) According to your result from part (c), what must the initial height of the drop be to produce at least 80 points?
- (e) According to your result from part (c), how many points are generated for a drop of mercury when  $h = 200$  cm? Assume  $r = 3.6$  mm,  $\rho = 13.5$  gm/cm<sup>3</sup>, and  $\sigma = 435$  dyn/cm.

**1.6.** The frequency  $\omega$  of waves on a deep ocean is found to depend on the wavelength  $\lambda$  of the wave, the surface tension  $\sigma$  of the water, the density  $\rho$  of the water, and gravity.

- (a) Use dimensional reduction to determine the functional dependence of  $\omega$  on  $\lambda$ ,  $\sigma$ ,  $\rho$ , and  $g$ .
- (b) In fluid dynamics it is shown that

$$\omega = \sqrt{gk + \frac{\sigma k^3}{\rho}},$$

where  $k = 2\pi/\lambda$  is the wavenumber. How does this differ from your result in (a)?

**1.7.** A ball is dropped from a height  $h_0$  and it rebounds to a height  $h_r$ . The rebound height depends on the elastic modulus  $E$ , radius  $R$ , and the mass density  $\rho$  of the ball. It also depends on the initial height  $h_0$  and the gravitational constant  $g$ .

- (a) Find a dimensionally reduced form for  $h_r$ .
- (b) Suppose it is found that  $h_r$  depends linearly on  $h_0$ , with  $h_r = 0$  if  $h_0 = 0$ . What does your formula from part (a) reduce to in this case?

- (c) Suppose the density of the ball is doubled. Use the result in (a) to explain how to change  $E$  so the rebound height stays the same.

**1.8.** A ball, when released underwater, will rise towards the surface with velocity  $v$ . This velocity depends on the density  $\rho_b$  and radius  $R$  of the ball, on gravity  $g$ , and on the density  $\rho_f$  and kinematic viscosity  $\nu$  of the water.

- (a) Find a dimensionally reduced form for  $v$ .  
 (b) In fluid mechanics, using Stokes' Law, it is found that

$$v = \frac{2gR^2(\rho_b - \rho_f)}{9\nu\rho_f}.$$

How does this differ from your result from part (a)? It is interesting to note that this formula is used by experimentalists to determine the viscosity of fluids. They do this by measuring the velocity in an apparatus called a falling ball viscometer, and then solving for  $\nu$  in the above formula.

**1.9.** In electric image tomography the objective is to determine the properties inside an object and this is done by applying a potential  $U$  to the surface. What is measured is the resulting electric current  $j$  on the surface. Suppose that it is found that the electric potential  $u$  within a spherical body depends on the object's radius  $R$  and conductivity  $\sigma$  as well as depends on  $U$  and  $j$ .

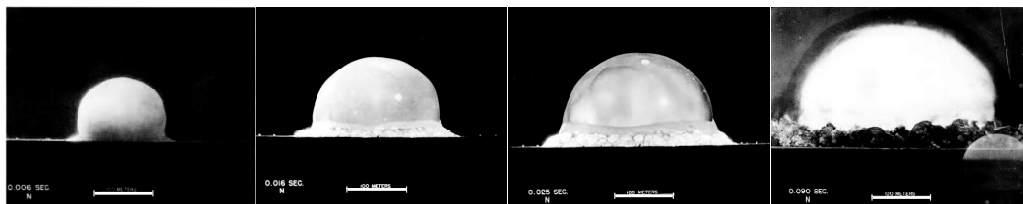
- (a) Find a dimensionally reduced form for  $u$ .  
 (b) Suppose that given a particular object that doubling the applied potential  $U$  causes the internal potential  $u$  to increase by a factor of four. How does this help simplify your result in (a)?  
 (c) Suppose it is necessary to know the internal potential  $u$  when using a large applied potential, say  $U = 2500V$ . However, for legal reasons it is required that only applied potentials less than  $250V$  can be used. Explain, using your result from (a), how to legally determine the large applied potential value.

**1.10.** The velocity  $v$  of water through a circular pipe depends on the pressure difference  $p$  between the two ends of the pipe, the length  $\ell$  and radius  $r$  of the pipe, as well as on the dynamic viscosity  $\mu$  and density  $\rho$  of the water.

- (a) Use dimensional analysis to determine the functional dependence of  $v$  on the above quantities.  
 (b) Suppose it is found that  $v$  depends linearly on  $p$ , with  $v = 0$  if  $p = 0$ . What does your formula from part (a) reduce to in this case?  
 (c) Your formula from part (b) should contain a general function of one, or more, dimensionless products. Explain how to experimentally determine this function. Be specific about which parameters are fixed, and which are varied, in the experiment. Also, your experiment should vary as few of the parameters as possible in determining this function.

**1.11.** In a high energy explosion there is a very rapid release of energy  $E$  that produces an approximately spherical shock wave that expands in time (Figure 1.10).





**Figure 1.10** Shock wave produced by a nuclear explosion, at 6 msec, 16 msec, 25 msec, and 90 msec. The width of the white bar in each figure is 100m (Brixner [2009]).

- Assuming the radius  $R$  of the shock wave depends on  $E$ , the length of time  $t$  since the explosion, and the density  $\rho$  of the air, use dimensional reduction to determine how the radius depends on these quantities. This expression is known as the Taylor-Sedov formula.
- It was shown by G. I. Taylor that if  $E = 1J$  and  $\rho = 1 kg/m^3$  then  $R = t^{2/5} m/s^{2/5}$ . Use this information and the result from (a) to find the exact formula for  $R$ .
- Use the photographs in Figure 1.10, and your result from (b) to estimate the energy released. The air density is  $\rho = 1 kg/m^3$ .
- The blast wave from a supernova can be modeled using the Taylor-Sedov formula. Explain how this can be used to estimate the date the supernova took place, using your result from part (b). As an example, use Tycho, which currently has a radius of about 33.2 light years, an estimated energy of  $10^{44}J$ , and density  $\rho = 2 \times 10^{-21} kg/m^3$ .

**1.12.** The vertical displacement  $u(x)$  of an elastic string of length  $\ell$  satisfies the boundary value problem

$$\tau \frac{d^2 u}{dx^2} + \mu u = p, \quad \text{for } 0 < x < \ell,$$

where  $u(0) = 0$ ,  $u(\ell) = U$ . Also,  $p$  is a constant and has the dimensions of force per length.

- What are the dimensions for the constants  $\tau$  and  $\mu$ ?
- Show how it is possible to nondimensionalize this problem so it takes the form

$$\frac{d^2 v}{ds^2} + \alpha v = \beta, \quad \text{for } 0 < s < 1,$$

where  $v(0) = 0$ ,  $v(1) = 1$ . Make sure to state what  $\alpha, \beta$  are.

**1.13.** From Newton's second law, the displacement  $y(t)$  of the mass in a mass, spring, dashpot system satisfies

$$m \frac{d^2 y}{dt^2} = F_s + F_d, \quad \text{for } 0 < t,$$

where  $m$  is the mass,  $F_s$  is the restoring force in the spring, and  $F_d$  is the damping force. To have a complete IVP we need to state the initial conditions,

and for this problem assume

$$y(0) = 0, \quad \frac{dy}{dt}(0) = v_0.$$

- (a) Suppose there is no damping, so  $F_d = 0$ , and the spring is linear, so  $F_s = -ky$ . What are the dimensions for the spring constant  $k$ ? Nondimensionalize the resulting IVP. Your choice for  $y_c$  and  $t_c$  should result in no dimensionless products being left in the IVP.
- (b) Now, in addition to a linear spring, suppose linear damping is included, so,

$$F_d = -c \frac{dy}{dt}.$$

What are the dimensions for the damping constant  $c$ ? Using the same scaling as in part (a), nondimensionalize the IVP. Your answer should contain a dimensionless parameter  $\epsilon$  that measures the strength of the damping. In particular, if  $c$  is small then  $\epsilon$  is small. The system in this case is said to have weak damping.

**1.14.** The velocity  $v(t)$  of the waves on a deep ocean satisfies the equation

$$\frac{dv}{dt} + kv^2 = \ell v, \quad \text{for } 0 < t,$$

where  $v(0) = V$ .

- (a) What are the dimensions of the constants  $k$ ,  $\ell$ , and  $V$ ?
- (b) Assuming a weak nonlinearity, use the Rules of Thumb given in Section 1.5 to nondimensionalize this problem.

**1.15.** The equation for an elastic beam is

$$EI \frac{\partial^4 u}{\partial x^4} + \rho \frac{\partial^2 u}{\partial t^2} = 0,$$

where the boundary conditions are  $u = u_0 \sin(\omega t)$  and  $\frac{\partial u}{\partial x} = 0$  at  $x = 0$ , while  $u = \frac{\partial u}{\partial x} = 0$  at  $x = \ell$ . Assume the initial conditions are  $u = 0$  and  $\frac{\partial u}{\partial t} = 0$  at  $t = 0$ . Here  $E$  is the elastic modulus,  $I$  is the moment of inertia, and  $\rho$  is the mass per unit length of the beam. Nondimensionalize the problem in such a way that the resulting boundary conditions contain no nondimensional groups.

**1.16.** When an end of a slender strip of paper is put into a cup of water, because of absorption, the water rises up the paper. The density  $\rho$  of the water along the strip satisfies the differential equation

$$\frac{\partial \rho}{\partial t} + \frac{\partial J}{\partial x} = 0,$$

where  $J$  is known as the flux.

- (a) What are the dimensions of  $J$ ?
- (b) The flux  $J$  depends on the gravitational constant  $g$ , the strip width  $d$ , the density gradient  $\frac{\partial \rho}{\partial x}$ , and the surface tension  $\sigma$  of the water. Find a dimensionally reduced form for  $J$ .
- (c) What does your result in (b) reduce to if it is found that  $J$  depends linearly on the density gradient, with  $J = 0$  if  $\rho_x = 0$ ? What is the resulting differential equation?
- (d) If the strip has length  $h$  the boundary conditions are  $\rho = \rho_0$  at  $x = 0$  and  $J = 0$  at  $x = h$ . The initial condition is  $\rho = 0$  at  $t = 0$ . With this, and your differential equation from (c), nondimensionalize the problem for  $\rho$  in such a way that no nondimensional groups appear in the final answer.

**1.17.** A thermokinetic model for the concentration  $u$  and temperature  $q$  of a mixture consists of the following equations (Gray and Scott [1994])

$$\begin{aligned}\frac{du}{dt} &= k_1 - k_2 u e^{k_3 q}, \\ \frac{dq}{dt} &= k_4 u e^{k_3 q} - k_5 q.\end{aligned}$$

The initial conditions are  $u = 0$  and  $q = 0$  at  $t = 0$ .

- (a) What are the dimensions of the  $k_i$ 's?
- (b) Explain why the rule of thumb for scaling used in the projectile problem does not help here.
- (c) Find the steady-state solution, that is, the solution of the differential equations with  $u' = 0$  and  $q' = 0$ .
- (d) Nondimensionalize the problem using the steady-state solution from (c) to scale  $u$  and  $q$ . Make sure to explain how you selected the scaling for  $t$ .

**1.18.** The equations that account for the relativistic motion of a planet around the sun are

$$\begin{aligned}\frac{d^2 r}{dt^2} - r \left( \frac{d\theta}{dt} \right)^2 &= -\frac{Gm}{r^2} + \frac{b}{r^3}, \\ \frac{d}{dt} \left( r^2 \frac{d\theta}{dt} \right) &= 0,\end{aligned}$$

where  $b$  is a constant. Assume the initial conditions are  $r = r_0$ ,  $r' = 0$ , and  $\theta = 0$  at  $t = 0$ .

- (a) What are the dimensions of  $r_0, b$ ?
- (b) Nondimensionalize the problem. The scaling should be chosen so the only nondimensional group appearing in the problem involves  $b$ .

**1.19.** Suppose you are given a dimensionless function  $f(\Pi)$  where  $\Pi$  is a dimensionless group. Also, suppose  $\Pi = A^a B^b C^c$  where  $A, B, C$  are dimensional parameters and the exponents  $a, b, c$  are nonzero numbers.

- (a) Show that if  $f(\Pi)$  is found to be linear in  $A$  then it must be that  $f(\Pi) = \alpha \Pi^{1/a} + \beta$  where  $\alpha, \beta$  are arbitrary numbers.
- (b) What can you conclude if it is found that  $\sqrt{AB}f(\Pi)$  is linear in  $A$ ?
- (c) Suppose it is found that if  $A$  is doubled that the value of  $F$  increases by a factor of four. Can this be used to determine  $F$ ?

**1.20.** This problem explores some consequences of dimensional quantities.

- (a) If  $g$  is the gravitational acceleration constant, explain why  $\sin(g)$  and  $e^g$  make no sense.
- (b) Explain why density, volume, and velocity can be used in place of length, mass, and time as fundamental units.
- (c) Explain why volume, velocity, and acceleration cannot be used in place of length, mass, and time as fundamental units.

**1.21.** In quantum chromodynamics three parameters that play a central role are the speed of light  $c$ , Planck's constant  $\hbar$ , and the gravitational constant  $G$ .

- (a) Explain why it is possible to use  $[c], [\hbar], [G]$  as fundamental units.
- (b) The distance  $\ell_p$  at which the strong, electromagnetic and weak forces become equal depends on  $c, \hbar, G$ . Find a dimensionally reduced form for how  $\ell_p$  depends on these three parameters. Based on this result, if the speed of light were to double what happens to  $\ell_p$ ?
- (c) The Bohr radius  $a$  of an electron depends on  $\hbar$ , the electron's charge  $e$ , and the mass  $m_e$  of the electron. Find a dimensionally reduced form for  $a$ .

**1.22.** The speed  $c_m$  at which magnetosonic waves travel through a plasma depends on the intensity  $B$  of the magnetic field, the permeability  $\mu_0$  of free space, and the density  $\rho$  and pressure  $p$  of the plasma.

- (a) Use dimensional reduction to determine the functional dependence of  $c_m$  on  $B, \mu_0, \rho$ , and  $p$ .
- (b) From the basic laws for plasmas it is shown that

$$c_m = \sqrt{V_A^2 + c_s^2},$$

where  $V_A = B/\sqrt{\mu_0\rho}$  is the Alfvén speed and  $c_s = \sqrt{\gamma p/\rho}$  is the sound speed in the gas. In the latter expression,  $\gamma$  is a number. How does this differ from your result in (a)?

**1.23.** In the study of the motion of particles moving along the  $x$ -axis one comes across the problem of finding the velocity  $u$  that satisfies the nonlinear partial differential equation

$$u_t + uu_x = 0, \tag{1.96}$$

where

$$u(x, 0) = \begin{cases} 0 & \text{if } x < 0 \\ u_0 & \text{if } 0 < x. \end{cases} \tag{1.97}$$

Assume that  $u_0$  is a positive constant. The equation (1.96) is derived in Chapter 5, and it is known as the inviscid Burgers' equation. It, along with the jump condition in (1.97), form what is known as a Riemann problem.

- What three physical quantities does  $u$  depend on?
- Use dimensional reduction, and a similarity variable, to reduce this problem to a nonlinear ordinary differential equation with two boundary conditions.
- Use the result from part (b) to solve the Riemann problem. The solution, which is known as an expansion fan, must be continuous for  $t > 0$ .
- What is the solution if the initial condition (1.97) is replaced with  $u(x, 0) = u_0$ ?
- Suppose that, rather than velocity, the variable  $u$  is displacement. Explain why it is not possible for  $u$  to satisfy (1.96).

**1.24.** Consider the partial differential equation

$$u_t + Du_{xxxx} = 0,$$

where  $u = u_0$  at  $x = 0$ ,  $u \rightarrow 0$  as  $x \rightarrow \infty$ , and  $u = 0$  at  $t = 0$ . Use dimensional reduction, and a similarity variable, to reduce this problem to an ordinary differential equation.

**1.25.** The equation of the concentration  $c$ , on an interval of length  $\ell$ , is

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} + \mu c,$$

where the boundary conditions are  $c(x, 0) = 0$ ,  $c(0, t) = c_0$ , and  $c(\ell, t) = 0$ .

- What are the dimensions of  $D$ ,  $c_0$ , and  $\mu$ ?
- Nondimensionalize the problem so it has the form

$$\frac{\partial u}{\partial s} = \frac{\partial^2 u}{\partial y^2} + \alpha u,$$

where the boundary conditions are  $u(y, 0) = 0$ ,  $u(0, s) = 1$ , and  $u(1, s) = 0$ .

**1.26.** One of the standard experimental tests used in the study of fluid motion through porous materials consists of determining the displacement  $u$  when the material is given a constant load. The governing differential equation in this case is

$$H \left[ 1 + \left( \frac{\partial u}{\partial x} \right)^3 \right] \frac{\partial^2 u}{\partial x^2} = \frac{\partial u}{\partial t}.$$

The boundary conditions are

$$\frac{\partial u}{\partial x} = -1, \quad \text{at } x = 0,$$

and

$$u = 0, \quad \text{as } x \rightarrow \infty.$$

The initial condition is

$$u = 0, \quad \text{at } t = 0.$$

- (a) What are the dimensions of the constant  $H$ ?
- (b) Find a dimensionally reduced form for the solution and then use this to transform the above diffusion problem into one involving a nonlinear ordinary differential equation. Make sure to state what happens to the boundary and initial conditions. You do not need to solve this problem.
- (c) In the experiment the surface displacement  $u(0, t)$  is measured. Without solving the problem use your results from (b) to sketch  $u(0, t)$  as a function of  $t$ .
- (d) Suppose the experimental data show that  $u(0, t) = 16t$  cm/sec. Using your result from part (c), explain why the mathematical model is incorrect. Also, explain why changing the differential equation to either  $Hu_{xx} = u_t$  or to  $H[1 + (u_x)^5]u_{xx} = u_t$  will also produce an incorrect model.

**1.27.** Consider the problem of solving the diffusion equation

$$D \frac{\partial^2 u}{\partial x^2} = \frac{\partial u}{\partial t},$$

where the boundary conditions are

$$u = 0, \quad \text{as } x \rightarrow \pm\infty.$$

Instead of an initial condition, assume the solution satisfies

$$\int_{-\infty}^{\infty} u dx = \gamma, \quad \forall t > 0.$$

- (a) What are the dimensions of  $\gamma$ ?
- (b) Find a dimensionally reduced form for the solution and then use this to transform the above diffusion equation into an ordinary differential equation. How do the boundary conditions transform? The integral condition should be considered in the dimensional reduction but its conversion using the similarity variable will wait until part (d).
- (c) Find the solution of the problem from part (b). You can assume  $F' \rightarrow 0$  and  $\eta F \rightarrow 0$  as  $\eta \rightarrow \pm\infty$ . As a hint, you might want to look for the expression  $(\eta F)'$  in your equation.
- (d) The solution from part (c) should contain an arbitrary constant. Find its value using the given integral condition and with this show that

$$u = \frac{\gamma}{\sqrt{\pi Dt}} e^{-x^2/(4Dt)}.$$

This is known as the fundamental, or point source, solution of the diffusion equation.

Introduction to the Foundations of Applied Mathematics

Holmes, M.H.

2009, XIV, 468 p., Hardcover

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Expert  
Solutions  
for Biopharma  
Applications

BIOPHARMA  
Pumps



Where Innovation Flows

MULTIPLE-USE QUATERNARY DIAPHRAGM PUMPS  
SINGLE-USE QUATERNARY DIAPHRAGM PUMPS

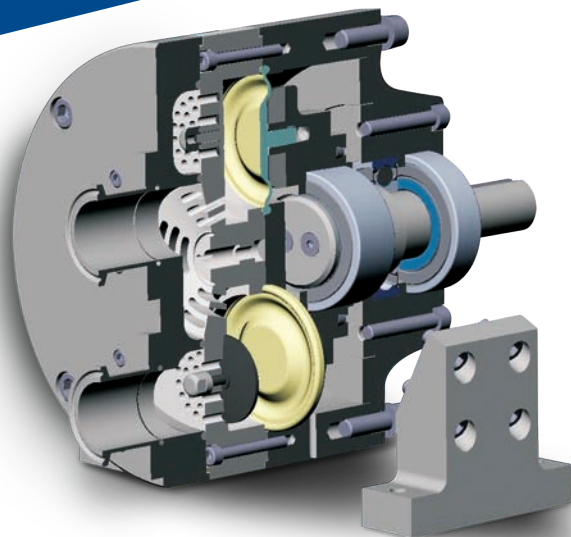


[quattroflow.com](http://quattroflow.com)





**QUATTROFLOW<sup>™</sup>**  
Fluid Systems



## The Idea: Pump Follows Evolution

How does a pump have to be designed to convey extremely delicate biologically active molecules? The solution is in nature itself!

Millions of years of evolution developed the perfect device to pump blood that contains albumin, gamma globulins, clotting factors and cells. It is the heart!

The Quattroflow displacement pump is based on this principle. The 4-piston (quaternary) diaphragm technology enables a gentle pumping through soft "heartbeats". Each stroke of the four diaphragms is generated by an eccentric shaft, which is connected to the electric motor.

The method of operation of Quattroflow pumps allows them to gently, safely and securely convey aqueous solutions and biologic products that are sensitive to shear force with minimal impact. The four-piston design does not require a mechanical seal or wetted rotating parts, ensuring total product containment without abrasion and minimum particle generation. Additionally, the four-piston pumping principle enables risk-free dry-running, low pulsation, self-priming and a high turn-down ratio.

# MARKETS SERVED

## BIOPHARMACEUTICAL:

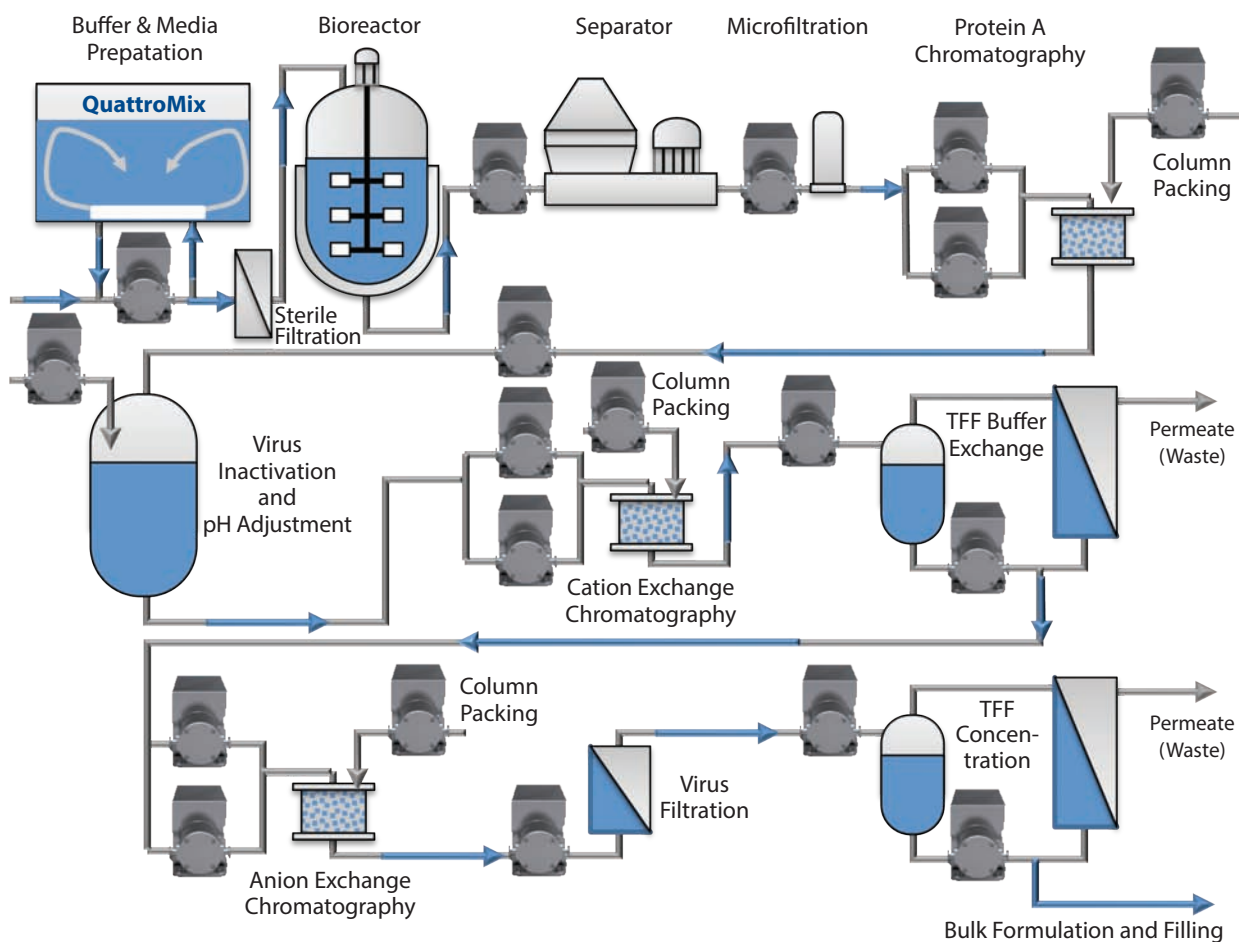
Quattroflow™ develops and manufactures single-use and multiple-use Quaternary (Four-Piston) Diaphragm Pumps for critical applications in the biopharmaceutical industry. This technology is CIP/SIP capable and offers disposable solutions that increase flexibility, reduce down-time, eliminate costs of cleaning validation, and risks of cross-contamination.

Quattroflow pumps can be found in all areas of biologic manufacturing such as cross-flow filtration systems, chromatography, and centrifuges. Quattroflow ensures safety, efficiency and reliability for handling biologics such as plasma products, therapeutic proteins, monoclonal antibodies, vaccines, and other high value products.

### Typical Applications and Products Handled:

- Chromatography systems
- Cross-flow systems, TFF
- Centrifuges
- Homogenizers
- Direct flow filters
- Reaction dosing
- Virus or sterile filtration
- Depth filtration
- Buffer mixing systems
- Blood plasma fractionation
- Virus cultures
- Bacterial and viral vaccines
- Cell cultures
- Cell cultures supernatants
- Enzyme solutions
- Antibodies
- Virus inactivation

### Quattroflow Pumps in the Up- and Downstream Processing of Monoclonal Antibodies





QF4400S  
Multiple-Use Quaternary  
Diaphragm Pump

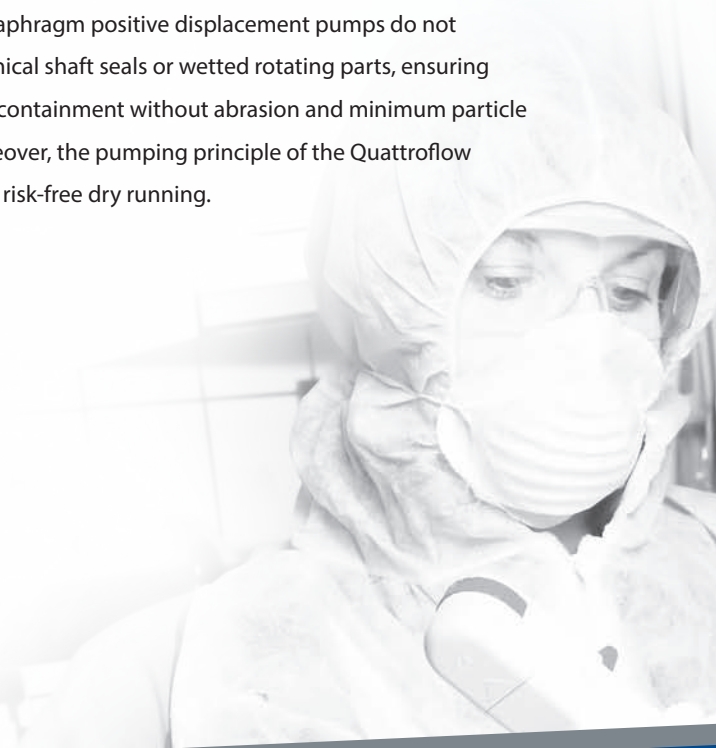
# Multiple-Use Pumps

## Pump Development for Special Applications

Biopharmaceutical processes require the highest level of purity, containment and cleanability. Quattroflow pumps meet these requirements as they are specially designed for such applications. Quattroflow provides the most versatile and efficient stainless-steel multiple-use pumps for worldwide applications in laboratories, pilot plants and production plants.

### QF-Series Multiple-Use Biopharma Pumps

The QF150S, QF1200S, QF4400S, QF5050S and QF20k Series quaternary diaphragm positive displacement pumps do not utilize mechanical shaft seals or wetted rotating parts, ensuring total product containment without abrasion and minimum particle creation. Moreover, the pumping principle of the Quattroflow pumps allows risk-free dry running.







# Single-Use Pumps

## The Single-Use Advantage

Quattroflow single-use pumps have a disposable product wetted chamber constructed of solid polypropylene (PP) or injection-molded polyethylene (PE) that can be replaced as a complete unit. The simple disposability of the pump chamber saves time and money by eliminating cleaning validation, sterilization and product cross-contamination. Single-use pumps are critical to reduce equipment turnaround times in the development biosimilar processes. In general, multi-product facilities is the typical field of application of the single-use pumps (e.g. process development, production of clinical reference samples, contract manufacturing).

## QF150SU, QF1200SU, QF4400SU and QF5050SU Single-Use Quaternary Diaphragm Pumps

The Quattroflow single-use pumps are self-priming and can be run dry. Inside the pump chamber, there are no rotating parts that are subject to friction that might cause particle generation and/or product modification/degradation due to heat generation. A single-use pump chamber can be replaced and ready for a new batch in a few minutes. Its simple design requires only one tool. An exchange kit is available to retrofit a multi-use pump into a single-use pump.



*PALL Allegro™ Single-Use Tangential Flow Filtration System" using a Quattroflow QF1200SU as recirculation pump.*



*PALL Allegro™ MVP Single-Use System" also with a Quattroflow QF1200SU pump, for different applications like virus filtration, sterile filtration, membrane chromatography, etc.*

*Images supplied courtesy of Pall Corporation*



QF150S  
Multiple-Use Quaternary  
Diaphragm Pump



QF1200SU  
Single-Use Quaternary  
Diaphragm Pump

#### TECHNOLOGY: 4-PISTON DIAPHRAGM

## Multiple-Use and Single-Use

### Quaternary Diaphragm Pumps

Quattroflow multiple-use pumps have a vast array of options and flow rates to accommodate many biopharmaceutical applications. Whether your requirement is 1 lph (0.017 lpm) or 20,000 lph (333 lpm) Quattroflow has your application covered with high purity, easily cleanable, multiple use units. From OEM's and small scale automated systems, to large laboratories and crossflow systems, the QF Series provides the purity needed for the most demanding pharmaceutical and bio-technology applications.

Quattroflow single-use combines convenience with the ability to save time and money by eliminating the cost of cleaning and decontamination. Gamma-irradiated upon request, these pumps ensure the integrity of your process and production output by providing the safe, clean and reliable transfer of your high purity process fluids.

#### Applications and Products

- Chromatography systems
- Cross-flow systems, TFF
- Centrifuges
- Homogenizers
- Direct flow filters
- Reaction dosing
- Virus or sterile filtration
- Depth filtration
- Buffer mixing systems
- Blood plasma fractionation
- Virus cultures
- Bacterial and viral vaccines
- Cell cultures
- Cell cultures supernatants
- Enzyme solutions
- Antibodies
- Virus inactivation
- DF/MF/UF filters

#### Features and Benefits

- Minimal maintenance
- Minimal downtime
- Low pulsation
- Superior containment
- Variable wide flow
- Capable of dry run
- Self-priming
- Cleanable outer surface
- Linear turndown
- Compact design
- Low heat input
- High purity
- Minimum particle shedding
- Quiet operation
- Lower life cycle cost
- Ease of use
- Quick start-up
- Scaleable
- No cell damage
- Low shear

#### Technical Data

- Stainless steel materials of construction
- Single-use pump chamber: Solid polypropylene or injection-molded polyethylene
- Valves: EPDM
- Diaphragm: TPE (EPDM/PP)

#### Performance Data

- Flow range: 1 lph - 20,000 lph (0.017 - 333 lpm)
- Max. discharge pressure: 6 bar (87 psi)
- Max. temperature: 130°C (266°F)







#### Certifications & Associations












# PUMP SELECTION GUIDE

## Multiple-Use Pumps

						
Pump Size	QF150S	QF1200S	QF1200S-CV	QF4400S	QF5050S	QF20k
Flow Range	1 – 180 lph 0.017 – 3 lpm	10 – 1,200 lph 0.167 – 20 lpm	10 – 1,200 lph 0.167 – 20 lpm	150 – 5,000 lph 2.5 – 83 lpm	50 – 5,000 lph 0.83 – 83 lpm	1,000 – 20,000 lph 16.7 – 333 lpm
More Data On Page	8	9	10	11	12	13

## Single-Use Pumps

							
Pump Size	QF150SU	QF1200SU	QF1200SU-M	QF1200SU-XAQ	QF1200SU-CV	QF4400SU	QF5050SU
Flow Range	1 – 180 lph 0.017 – 3 lpm	10 – 1,200 lph 0.167 – 20 lpm	10 – 1,200 lph 0.167 – 20 lpm	10 – 1,200 lph 0.167 – 20 lpm	10 – 1,200 lph 0.167 – 20 lpm	150 – 5,000 lph 2.5 – 83 lpm	50 – 5,000 lph 0.83 – 83 lpm
More Data On Page	14	15	16	17	18	19	20

For Accessories please see page 21.

All Quattroflow pump sizes are available with different options, such as controller, housing, ports and motor. Consider the standard versions of all sizes for the technical data presented on the following pages.



# QF150S

## Quaternary Diaphragm Pumps

### Multiple-Use

- Integrated controller
- Digital key pad for manual operation
- Small and portable format
- Ideal for R&D and process development

### Technical Data

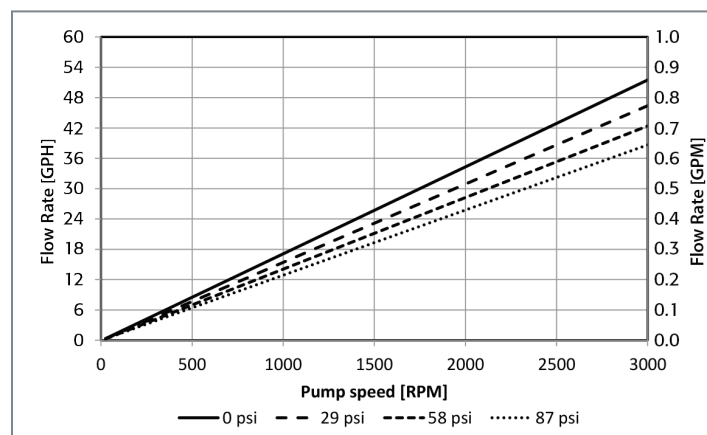
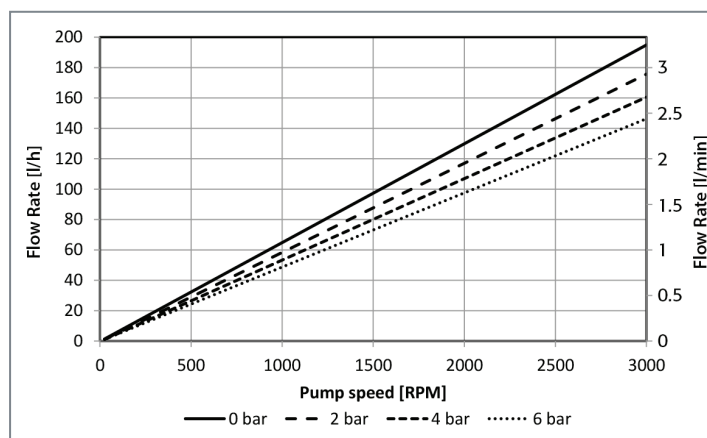
QF150S Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 5°	180 lph (3 lpm)
Flow Rate Minimum:	Eccentric Shaft 5°	1 lph (0.017 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	6 bar (87 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	80° C (176° F)
	CIP	90° C (194° F)
	SIP	130° C (266° F)
	Autoclave	130° C (266° F)
Suction Lift Dry at 3000 rpm:	Eccentric Shaft 5°	2 - 3 m (6.6 - 9.8 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	1.2 ml
	Filling Volume Without Connectors	15 ml
Connection Specification (Standard):	Connectors	1/4" TC
	Position of Connectors	Inline
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Housing	SS316L
	Valve Plate	SS316L
	Diaphragms	TPE
	Valves	EPDM
	O-rings	EPDM
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI;
	Stainless Steel Parts (product wetted)	FDA21CFR177; BSE/TSE Safe 3.1; Surface Roughness; Ferrite Content
Motor (Standard):	Rated speed	3000 min <sup>-1</sup>
	Voltage	230 V (110 V as option)
	Power	0.05 KW
Pump Dimension with Motor and Housing:	Length	280.5 mm (11.04")
	Width	115 mm (4.53")
	Height	184 mm (7.24")
Pump Weight with Motor and Housing:		8.4 kg (19 lb.)

Other connection specifications, materials and motors available on request.



### Performance Charts

#### Eccentric Shaft: 5°

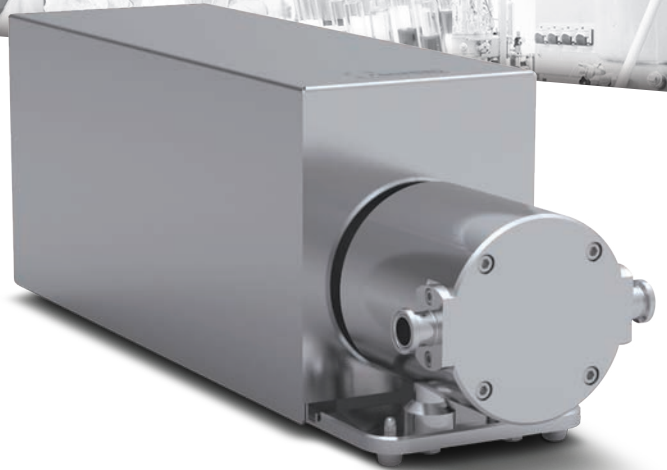


# QF1200S

## Quaternary Diaphragm Pumps

### Multiple-Use

- Separate control box for manual operation available
- ATEX version available

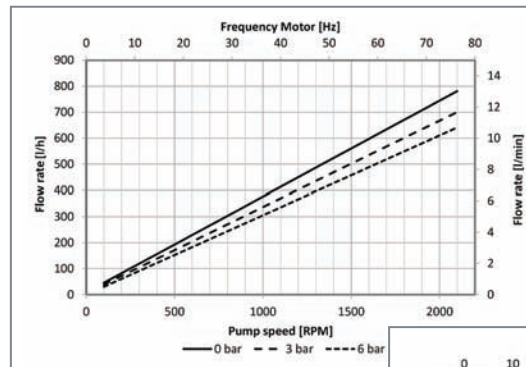


## Technical Data

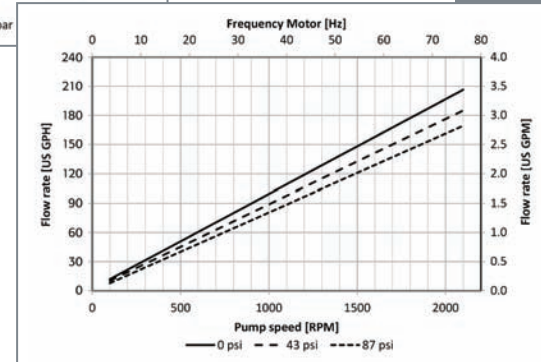
QF1200S Standard Motor		
Flow Rate	Eccentric Shaft 3°	800 lph (13.3 lpm)
	Maximum: Eccentric Shaft 5°	1200 lph (20 lpm)
Flow Rate Minimum*	Eccentric Shaft 3°	10 lph (0.167 lpm)
	Eccentric Shaft 5°	20 lph (0.333 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	6 bar (87 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	80° C (176° F)
	CIP	90° C (194° F)
	SIP	130° C (266° F)
	Autoclave	130° C (266° F)
Pump Speed Range:	rpm	30 - 2,400
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 3°	2.5 - 3 m (8.2-9.8 ft)
	Eccentric Shaft 5°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	9.6 ml (5°)
	Filling Volume Without Connectors	75 ml
Connection Specification (Standard):	Connectors	3/4" TC
	Position of Connectors	Inline
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Housing	SS316L
	Valve Plate	SS316L
	Diaphragms	TPE
	Valves	EPDM
Certificates/ Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe
	Stainless Steel Parts (product wetted)	3.1; Surface Roughness; Ferrite Content
Motor (Standard):	Rated speed	1375 min-1 (50 Hz)
	Voltage	230/400 V
	Power	0.37 KW
Pump Dimension with Motor and Housing:	Length	487 mm (19.17")
	Width	159 mm (6.26")
	Height	210 mm (8.27")
Pump Weight with Motor and Housing:		24 kg (53 lb.)

Other connection specifications, materials and motors available on request.

\* When using pump with control box: 20 lph (0.333 lpm) and 40 lph (0.667 lpm)

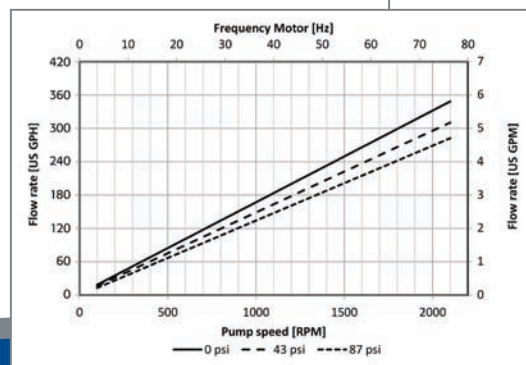
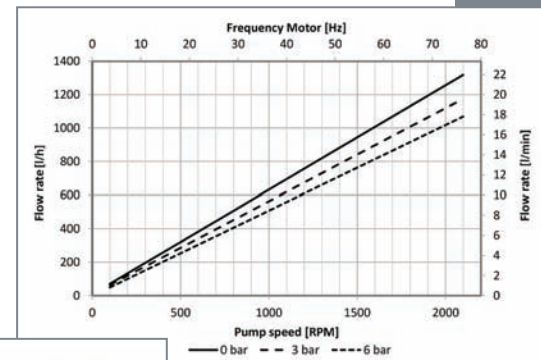


Eccentric Shaft: 3°



## Performance Charts

Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.



Eccentric Shaft: 5°



# QF1200S-CV

## Quaternary Diaphragm Pumps

### Multiple-Use

- Integrated controller
- Digital key pad for manual operation
- Compact size
- 230V motor

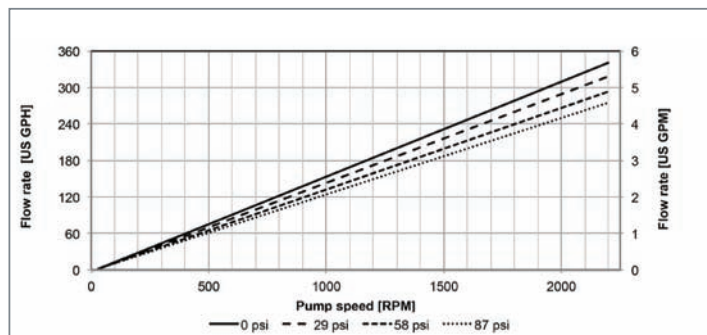
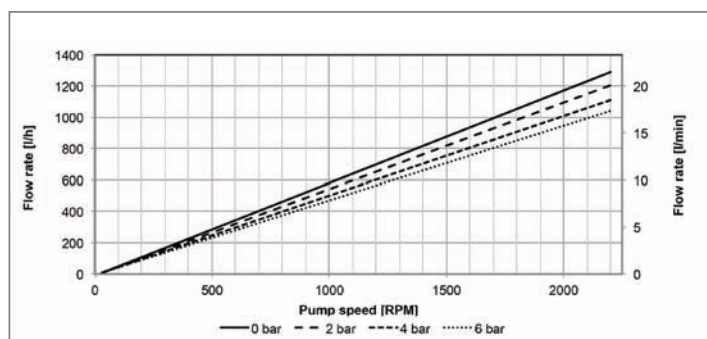
### Technical Data

QF1200S-CV Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 5°	1200 lph (20 lpm)
Flow Rate Minimum:	Eccentric Shaft 5°	10 lph (0.167 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	6 bar (87 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	80° C (176° F)
	CIP	90° C (194° F)
	SIP	130° C (266° F)
	Autoclave	130° C (266° F)
Pump Speed Range:	rpm	10 - 2200
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 5°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Filling Volume Without Connectors	75 ml
Connection Specification (Standard):	Connectors	3/4" TC
	Position of Connectors	Inline
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Chamber	SS316L
	Valve Plate	SS316L
	Diaphragms	TPE
	Valves	EPDM
Certificates/Proofs (Optional):	O-rings	EPDM
	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe
Motor:	Stainless Steel Parts (product wetted)	3.1; Surface Roughness; Ferrite Content
	Rated speed	2200 min-1
	Voltage	230 V
Pump Dimension with Motor and Housing:	Power	0.75 kW
	Length	487 mm (19.17")
	Width	200 mm (7.87")
Pump Weight with Motor and Housing:	Height	210 mm (8.27")
		25 kg (55 lb.)



### Performance Charts

#### Eccentric Shaft: 5°



# QF4400S

## Quaternary Diaphragm Pumps

### Multiple-Use

- Separate control box for manual operation available
- ATEX version available

### Technical Data

QF4400S Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 6°	5000 lph (83 lpm)
Flow Rate Minimum:	Eccentric Shaft 6°	150 lph (2.5 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	6 bar (87 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	80° C (176° F)
	CIP	90° C (194° F)
	SIP	130° C (266° F)*
	Autoclave	130° C (266° F)*
Suction Lift Dry at 1200 rpm:	Eccentric Shaft 6°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	95 ml
	Filling Volume Without Connectors	820 ml
Connection Specification (Standard):	Connectors	1.5" TC
	Position of Connectors	Front
Product Wetted Materials (Standard):	Pump Housing	SS316L
	Valve Plate	SS316L or PP
	Diaphragms	TPE
	Valves	EPDM/SS316L
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe
	Stainless Steel Parts (product wetted)	3.1; Surface Roughness; Ferrite Content
Motor (Standard):	Rated speed	1410 min-1 (50 Hz)
	Voltage	230/400 V
	Power	2.2 KW
Pump Dimension with Motor and Housing:	Length	776.4 mm (30.57")
	Width	256 mm (10.08")
	Height	330 mm (12.99")
Pump Weight with Motor and Housing:		96 kg (212 lb.)

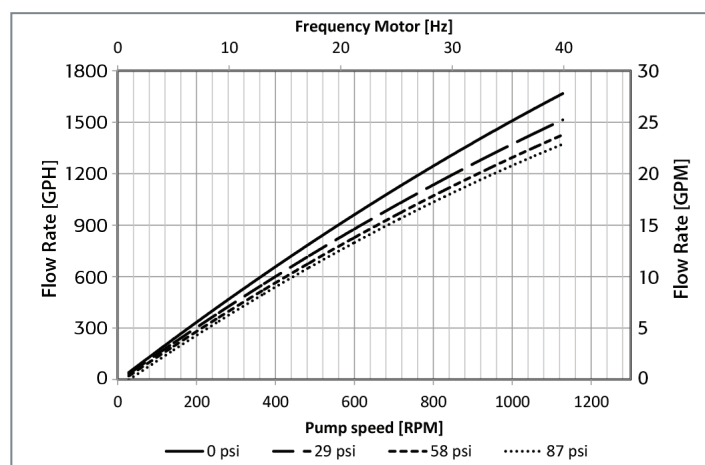
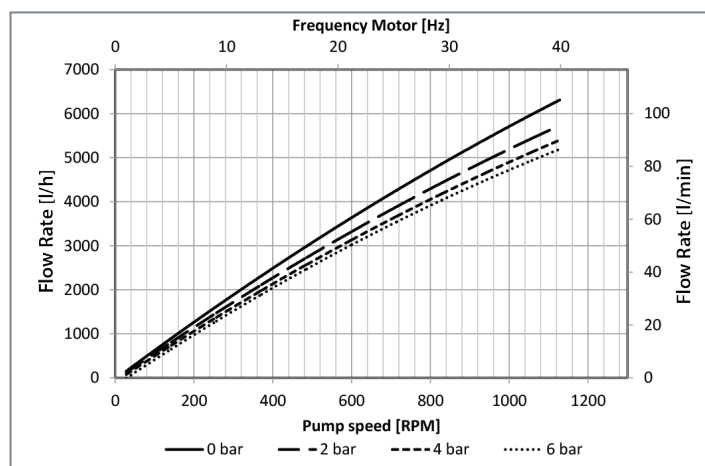
Other connection specifications, materials and motors available on request.

\*With SS316L valve plate only



### Performance Charts

#### Eccentric Shaft: 6°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.

# QF5050S

## Quaternary Diaphragm Pumps

### Multiple-Use

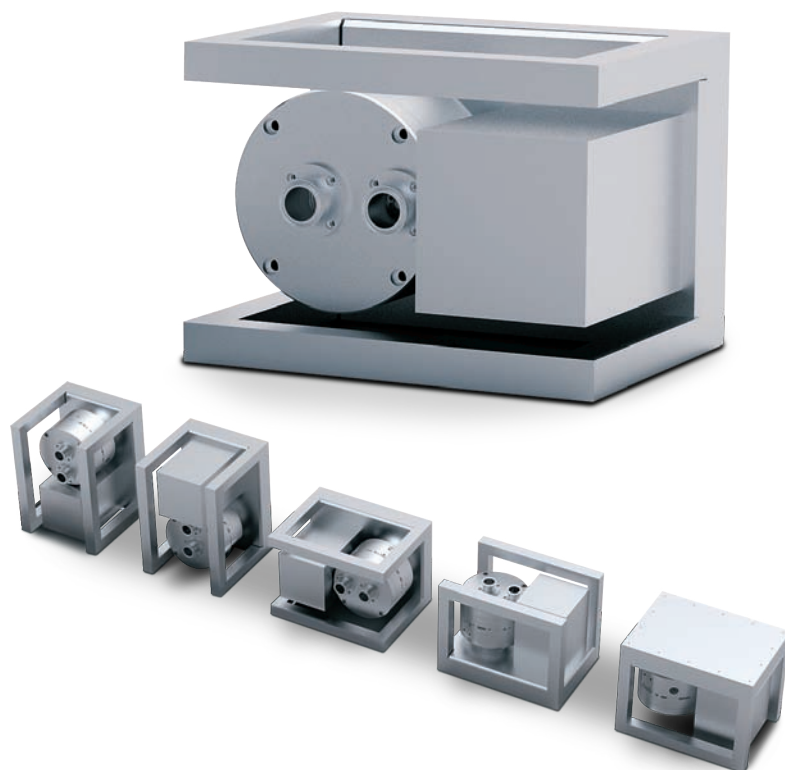
- Compact footprint
- High turn-down ratio
- Multi-option installation flexibility
- Separate control box for manual operation available

### Technical Data

QF5050S Servo Motor		
Flow Rate Maximum:	Eccentric Shaft 6°	5000 lph (83 lpm)
Flow Rate Minimum:	Eccentric Shaft 6°	50 lph (0.83 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	6 bar (87 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	80° C (176° F)
	CIP	90° C (194° F)
	SIP	130° C (266° F)*
	Autoclave	130° C (266° F)*
Suction Lift Dry at 1200 rpm:	Eccentric Shaft 6°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	95 ml
	Filling Volume Without Connectors	820 ml
Connection Specification (Standard):	Connectors	1.5" TC
	Position of Connectors	Front
Product Wetted Materials (Standard):	Pump Housing	SS316L
	Valve Plate	SS316L or PP
	Diaphragms	TPE
	Valves	EPDM/SS316L
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe
	Stainless Steel Parts (product wetted)	3.1; Surface Roughness; Ferrite Content
Motor (Standard):	Rated speed	3000 min <sup>-1</sup> (2.66:1 reduction)
	Voltage	400 V
	Power	3 KW
Pump Dimension with Motor and Housing:	Length	440 mm (17.32")
	Width	325 mm (12.80")
	Height	320 mm (12.60")
Pump Weight with Motor and Housing:		66 kg (146 lb.)

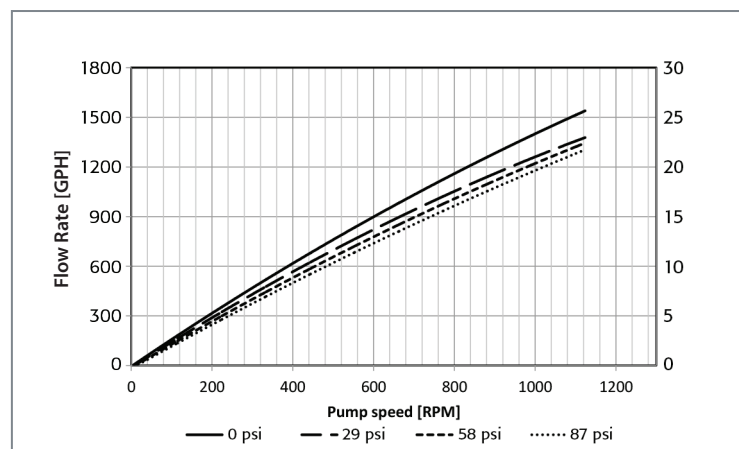
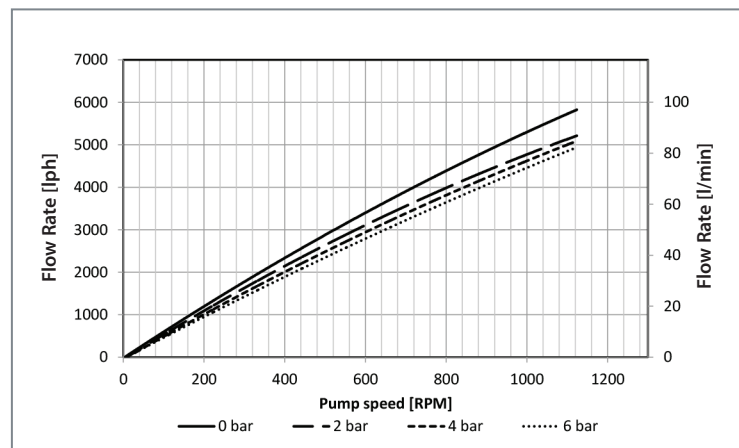
Other connection specifications, materials and motors available on request.

\*With SS316L valve plate only



### Performance Charts

#### Eccentric Shaft: 6°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.

# QF20k

## Quaternary Diaphragm Pumps

### Multiple-Use

- Separate control box for manual operation available
- ATEX version available

### Technical Data

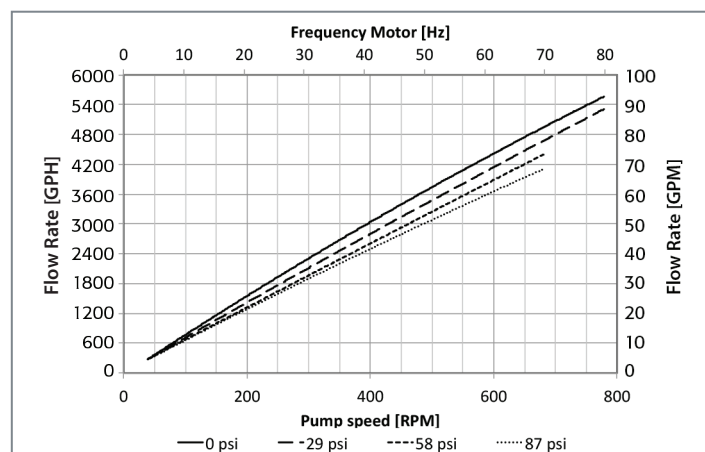
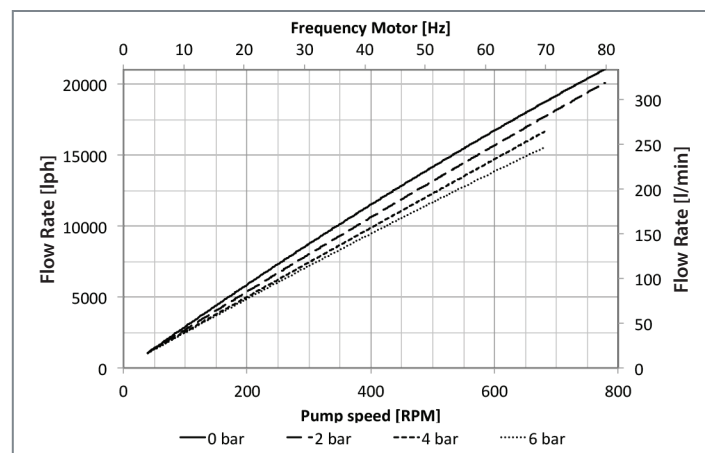
QF20k Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 7°	20000 lph (333 lpm)
Flow Rate Minimum:	Eccentric Shaft 7°	1000 lph (16.7 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	6 bar (87 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	80° C (176° F)
	CIP	90° C (194° F)
	SIP	130° C (266° F)
	Autoclave	130° C (266° F)
Suction Lift Dry at 330 rpm:	Eccentric Shaft 7°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	470 ml
	Filling Volume Without Connectors	2950 ml
Connection Specification (Standard):	Connectors	2" TC
	Position of Connectors	Front
Product Wetted Materials (Standard):	Pump Housing	SS316L
	Valve Plate	SS316L
	Diaphragms	TPE
	Valves	EPDM/SS316L
Certificates/Proofs (Optional):	O-rings	EPDM
	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe
Stainless Steel Parts (product wetted)		3.1; Surface Roughness; Ferrite Content
	Rated speed	1460/474 min-1 (50 Hz)
Motor (Standard):	Voltage	230/400 V
	Power	4 KW
Pump Dimension with Motor and Housing:	Length	1152.5 mm (45.37")
	Width	400 mm (15.75")
	Height	416 mm (16.38")
Pump Weight with Motor and Housing:		217 kg (478 lb.)

Other connection specifications, materials and motors available on request.



### Performance Charts

#### Eccentric Shaft: 7°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.



# QF150SU

## Quaternary Diaphragm Pumps

### Single-Use

- Disposable wetted product chamber
- Integrated controller
- Digital key pad for manual operation
- Small and portable format
- Ideal for R&D and process development

### Technical Data

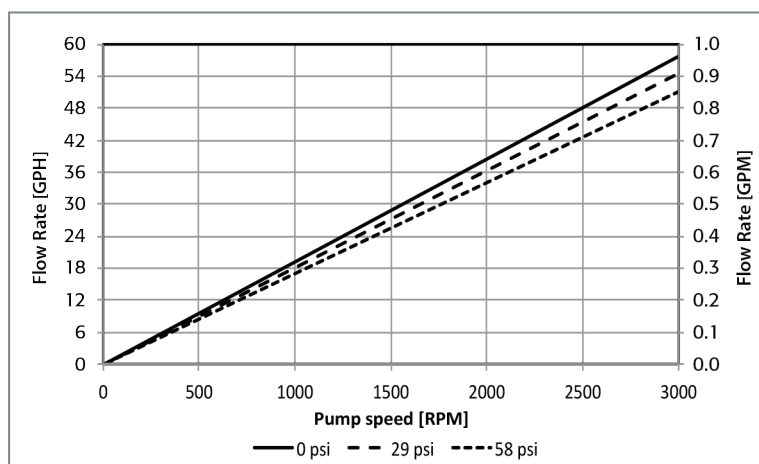
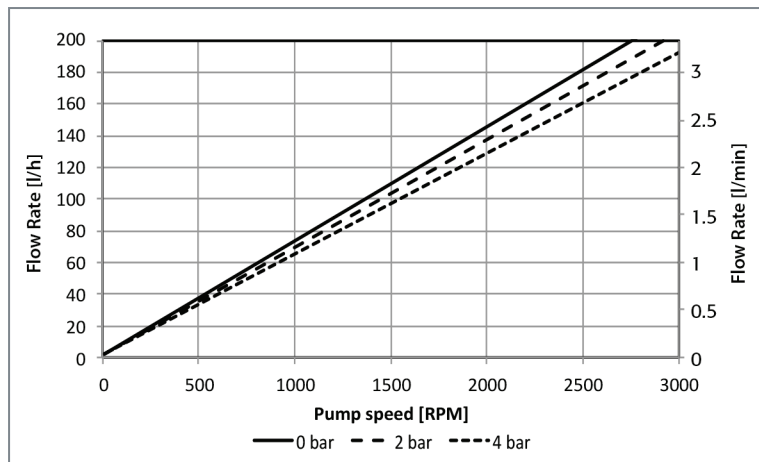
QF150SU Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 5°	180 lph (3 lpm)
Flow Rate Minimum:	Eccentric Shaft 5°	1 lph (0.017 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	4 bar (58 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	60° C (140° F)
	Autoclave*	130° C (266° F)
Suction Lift Dry at 3000 rpm:	Eccentric Shaft 5°	2 - 3 m (6.6 - 9.8 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	1.2 ml
	Filling Volume Without Connectors	15 ml
Connection Specification (Standard):	Connectors	1/4" TC
	Position of Connectors	Inline
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Chamber	PP
	Valve Plate	PP
	Diaphragms	TPE
	Valves	EPDM
	O-rings	EPDM
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe; USP 87/381/661
Motor (Standard):	Rated speed	3000 min-1
	Voltage	230 V (110 V as option)
	Power	0.05 KW
Pump Dimension with Motor and Housing:	Length	285 mm (11.22")
	Width	115 mm (4.53")
	Height	184 mm (7.24")
Pump Weight with Motor and Housing:		7 kg (15.8 lb.)

Other connection specifications, materials and motors available on request.



### Performance Charts

#### Eccentric Shaft: 5°

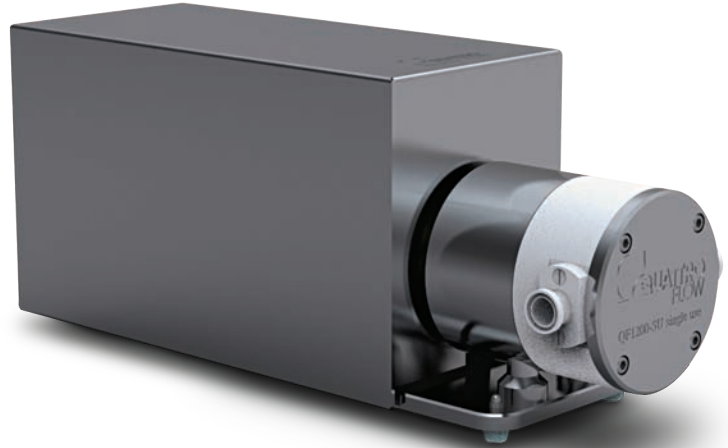


# QF1200SU

## Quaternary Diaphragm Pumps

### Single-Use

- Disposable wetted product chamber
- Pump chamber made of solid polypropylene
- Separate control box for manual operation available
- ATEX version available



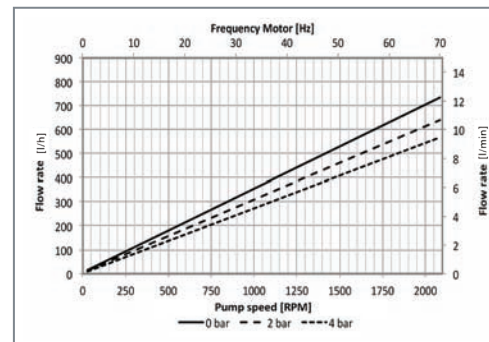
### Technical Data

QF1200SU Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 3°	800 lph (13.3 lpm)
	Eccentric Shaft 5°	1200 lph (20 lpm)
Flow Rate Minimum*:	Eccentric Shaft 3°	10 lph (0.167 lpm)
	Eccentric Shaft 5°	20 lph (0.333 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	4 bar (58 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	60° C (140° F)
	Autoclave	130° C (266° F)
Pump Speed Range:	rpm	30 - 2,400
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 3°	2.5 - 3 m (8.2-9.8 ft)
	Eccentric Shaft 5°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	9.6 ml (5°)
	Filling Volume Without Connectors	5.8 ml (3°)
Connection Specification (Standard):	Connectors	3/4" TC
	Position of Connectors	Inline
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Chamber	PP
	Valve Plate	PP
	Diaphragms	TPE
	Valves	EPDM
Certificates/Proofs (Optional):	Elastomere (product wetted)	EPDM
		USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe; USP 87/381/661
Motor (Standard):	Rated speed	1375 min-1 (50 Hz)
	Voltage	230/400 V
	Power	0.37 KW
Pump Dimension with Motor and Housing:	Length	497 mm (19.56")
	Width	159 mm (6.26")
	Height	210 mm (8.27")
Pump Weight with Motor and Housing:		21 kg (46 lb.)

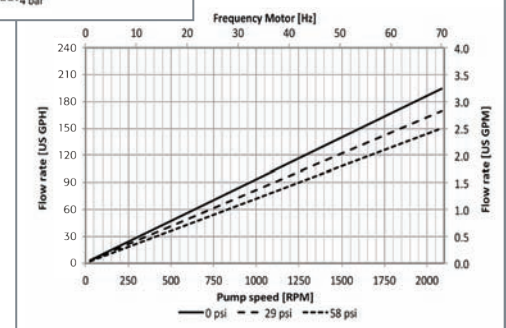
Other connection specifications, materials and motors available on request.

\* When using pump with control box: 20 lph (0.333 lpm) and 40 lph (0.667 lpm)

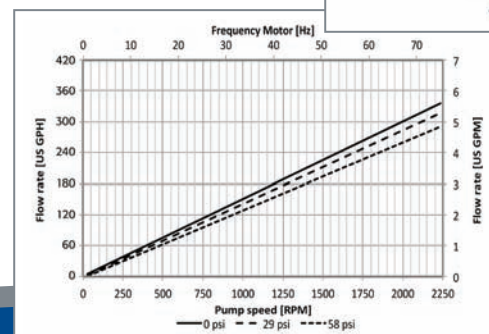
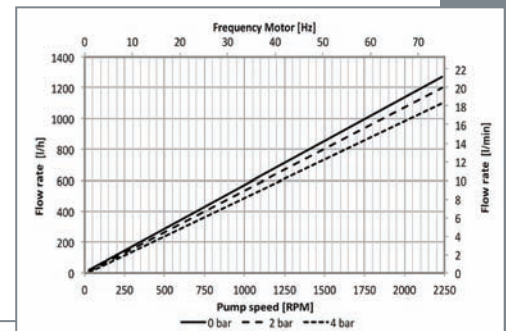
### Performance Charts



#### Eccentric Shaft: 3°



#### Eccentric Shaft: 5°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.

# QF1200SU-M

## Quaternary Diaphragm Pumps

### Single-Use

- Disposable wetted product chamber
- Pump chamber made of injection-molded polyethylene
- Separate control box for manual operation available
- ATEX version available
- Front side connections

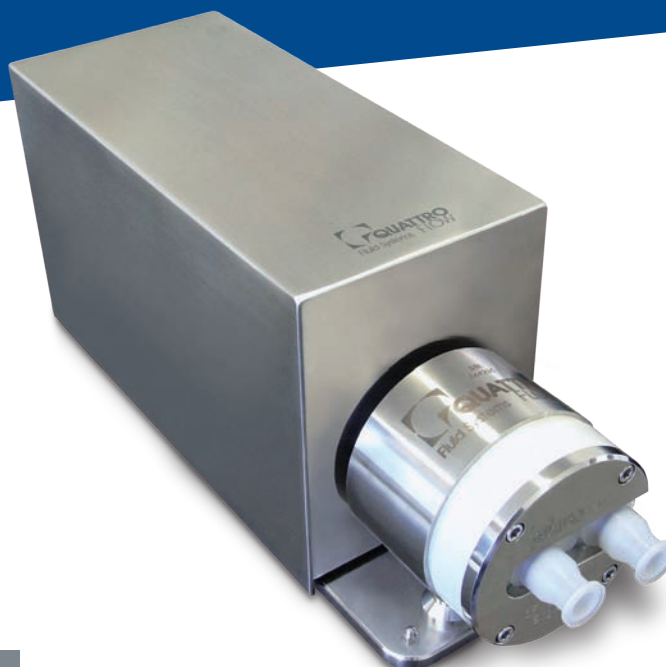
### Technical Data

QF1200SU-M Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 3°	800 lph (13.3 lpm)
	Eccentric Shaft 5°	1200 lph (20 lpm)
Flow Rate Minimum*:	Eccentric Shaft 3°	10 lph (0.167 lpm)
	Eccentric Shaft 5°	20 lph (0.333 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	4 bar (58 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	50° C (122° F)
Pump Speed Range:	rpm	30 - 2,400
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 3°	2-2.5 m (6.6-8.2 ft)
	Eccentric Shaft 5°	3-3.5 m (9.8-11.5 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	9.6 ml (5°) 5.8 ml (3°)
	Filling Volume Without Connectors	75 ml
Connection Specification (Standard):	Connectors	3/4" TC
	Position of Connectors	Front
Product Wetted Materials (Standard):	Pump Chamber	PE injection molded**
	Valve Plate	PE injection molded
	Diaphragms	TPE
	Valves	EPDM
Certificates/Proofs (Optional):	Elastomere (product wetted)	EPDM
		USP <88> Cl. VI; FDA 21 CFR 177; BSE/TSE Safe; USP 87/381/661
	Rated speed	1375 min <sup>-1</sup> (50 Hz)
	Voltage	230/400 V
Motor (Standard):	Power	0.37 KW
	Length	503 mm (19.8")
	Width	159 mm (6.26")
Pump Dimension with Motor and Housing:	Height	210 mm (8.27")
	Pump Weight with Motor and Housing:	20 kg (44 lb.)

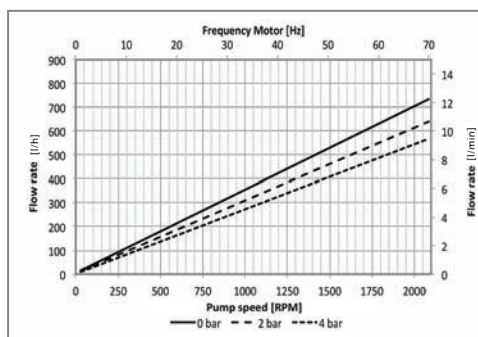
Other connection specifications, materials and motors available on request.

\* When using pump with control box: 20 lph (0.333 lpm) and 40 lph (0.667 lpm)

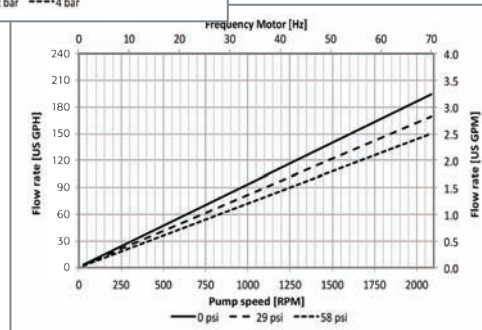
\*\* Connectors PP



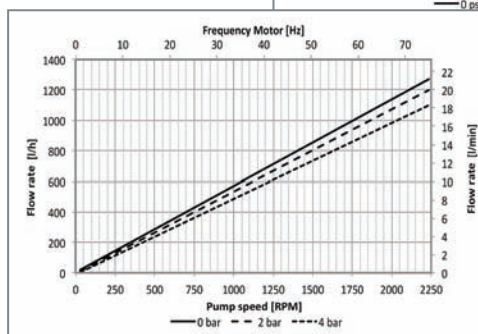
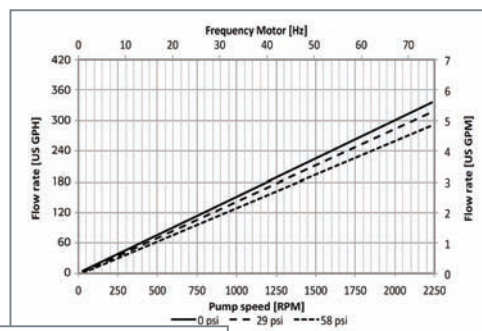
### Performance Charts



#### Eccentric Shaft: 3°



#### Eccentric Shaft: 5°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.

# QF1200SU-XAQ

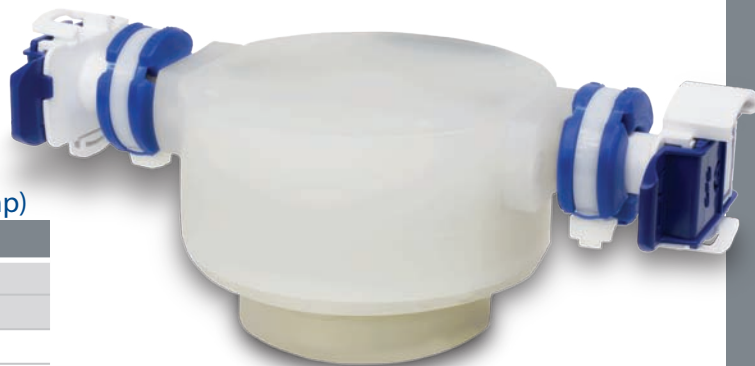
## Pre-Gamma-Irradiated Single-Use Pump Chamber with CPC™ AseptiQuik® Connector

The use of aseptic connectors of CPC enables simple and flexible aseptic integration of gamma irradiated Quattroflow single-use pump chambers in biopharmaceutical processes. Depending on the process tubing requirements it allows users to flexibly and aseptically connect the pump chambers with bags, tubing and filters, which are also equipped with matching aseptic connectors.

- Disposable wetted product chamber
- Pre-gamma-irradiated
- Fast & easy aseptic integration
- Easy replacement

### Technical Data with Standard Drive (QF1200SU Pump)

QF1200SU-XAQ Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 3°	800 lph (13.3 lpm)
	Eccentric Shaft 5°	1200 lph (20 lpm)
Flow Rate Minimum*:	Eccentric Shaft 3°	10 lph (0.167 lpm)
	Eccentric Shaft 5°	20 lph (0.333 lpm)
Pressure:	Temperature of Fluid ≤ 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	40° C (104° F)
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 3°	2.5 - 3 m (8.2 - 9.8 ft)
	Eccentric Shaft 5°	4 - 4.5 m (13.1 - 14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	5.8 ml (3°) 9.6 ml (5°)
	Filling Volume Without Connectors	75 ml
Connection Specification (Standard):	Connectors	AseptiQuik® G
	Sanitary Clamp	Sanisure® 3/4"
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Chamber / Valve Plate	PP
	Pump Diaphragms	TPE
	Pump Valves	EPDM
	Valves / O-rings	EPDM
	Connector Seals / TC Gasket	Platinum-Cured Silicone
	Connector Main Components	Polycarbonate
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe
Gamma Irradiation:	Gamma dose	25 kGy
	Certificate	Included
	Comment	Double bagged package



Other connection specifications, materials and motors available on request.

\* When using pump with control box: 20 lph (0.333 lpm) and 40 lph (0.667 lpm)

For the performance charts, please refer to the QF1200SU pump specifications on page 15.



# QF1200SU-CV

## Quaternary Diaphragm Pumps

### Single-Use

- Disposable wetted product chamber
- Integrated controller
- Digital key pad for manual operation
- Compact size

### Technical Data

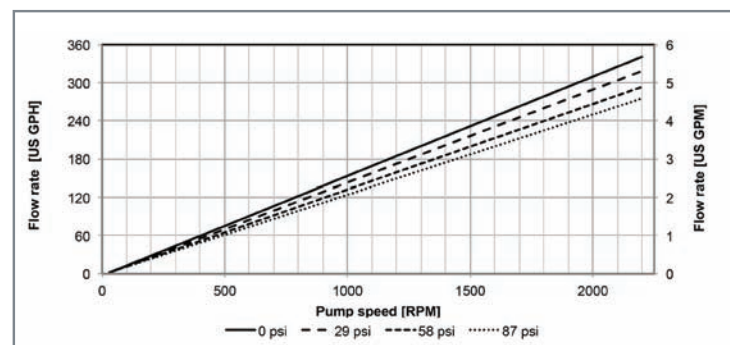
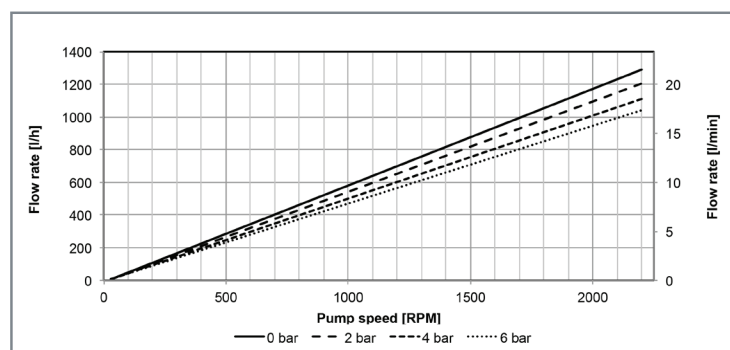
QF1200SU-CV		
Flow Rate Maximum:	Eccentric Shaft 5°	1200 lph (20 lpm)
Flow Rate Minimum:	Eccentric Shaft 5°	10 lph (0.167 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	4 bar (58 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	60° C (140° F)
	Autoclave	130° C (266° F)
Pump Speed Range:	rpm	10 - 2200
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 5°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	9.6 ml
	Filling Volume Without Connectors	75 ml
Connection Specification (Standard):	Connectors	3/4" TC
	Position of Connectors	Inline
	Number of Flow Directions	4
Product Wetted Materials (Standard):	Pump Chamber	PP
	Valve Plate	PP
	Diaphragms	TPE
	Valves	EPDM
	O-rings	EPDM
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe; USP 87/381/661
Motor:	Rated speed	2200 min <sup>-1</sup>
	Voltage	230 V
	Power	0.75 kW
Pump Dimension with Motor and Housing:	Length	497 mm (19.56")
	Width	200 mm (7.87")
	Height	210 mm (8.27")
Pump Weight with Motor and Housing:		21 kg (46 lb.)

Technical data for the QF1200SU-CV-M (pump chamber made of injection-molded PE) available on request.



### Performance Charts

#### Eccentric Shaft: 5°



# QF4400SU

## Quaternary Diaphragm Pumps

### Single-Use

- Disposable wetted product chamber
- Separate control box for manual operation available
- ATEX version available

### Technical Data

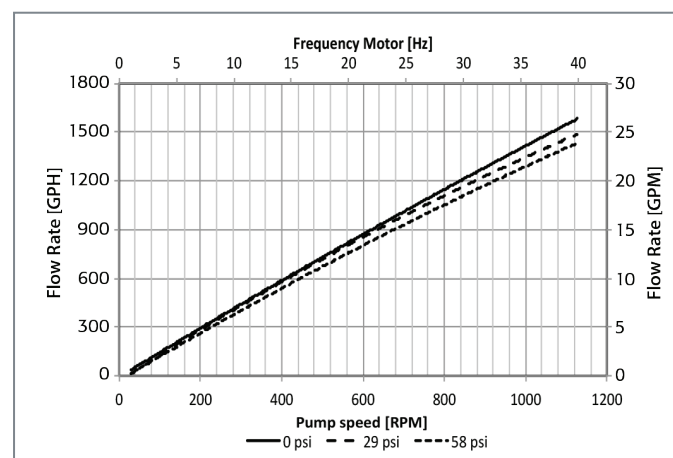
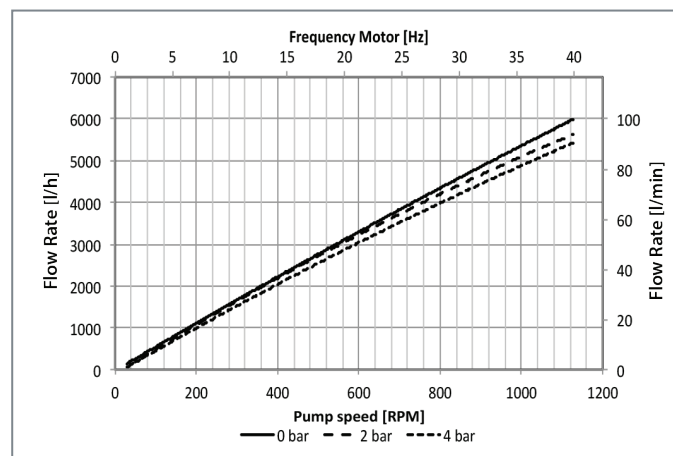
QF4400SU Standard Motor		
Flow Rate Maximum:	Eccentric Shaft 6°	5000 lph (83 lpm)
Flow Rate Minimum:	Eccentric Shaft 6°	150 lph (2.5 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	4 bar (58 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	60° C (140° F)
	Autoclave	130° C (266° F)
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 6°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	95 ml
	Filling Volume Without Connectors	820 ml
Connection Specification (Standard):	Connectors	1.5" TC
	Position of Connectors	Front
Product Wetted Materials (Standard):	Pump Chamber	PP
	Valve Plate	PP
	Diaphragms	TPE
	Valves	EPDM / SS316L
Certificates/Proofs (Optional):	O-rings	EPDM
	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe; USP 87/381/661
Motor:	Rated speed	1410 min-1 (50 Hz)
	Voltage	230/400 V
	Power	2.2 KW
Pump Dimension with Motor and Housing:	Length	776.4 mm (30.57")
	Width	256 mm (10.08")
	Height	330 mm (12.99")
Pump Weight with Motor and Housing:		81 kg (178 lb.)

Other connection specifications, materials and motors available on request.



### Performance Charts

#### Eccentric Shaft: 6°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.

# QF5050SU

## Quaternary Diaphragm Pumps

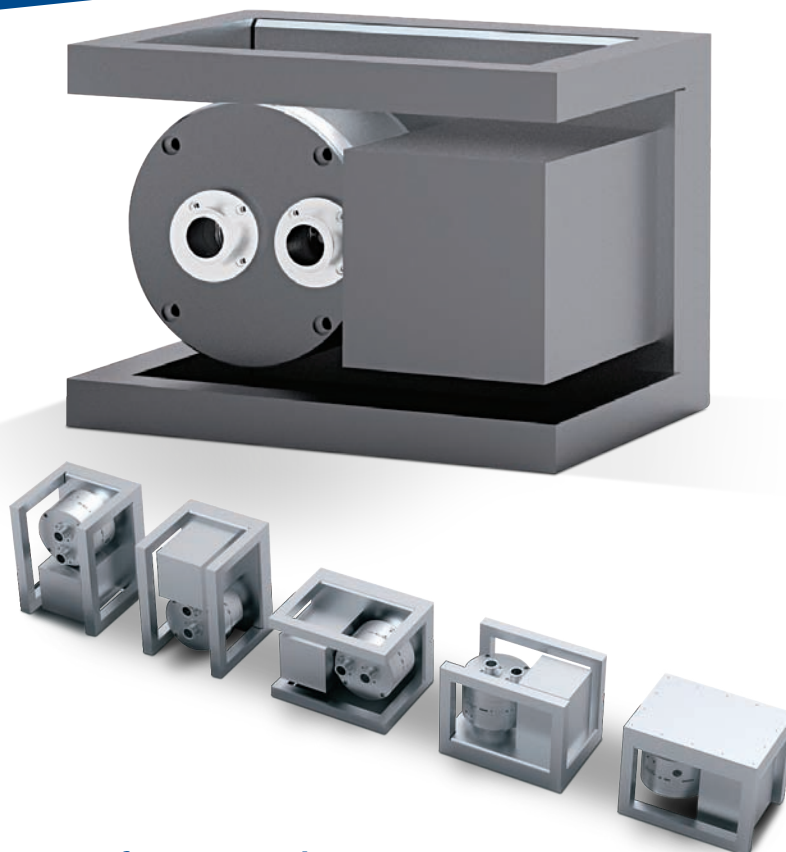
### Single-Use

- Disposable wetted product chamber
- Compact footprint
- High turn-down ratio
- Multi-option installation flexibility
- Separate control box for manual operation available

### Technical Data

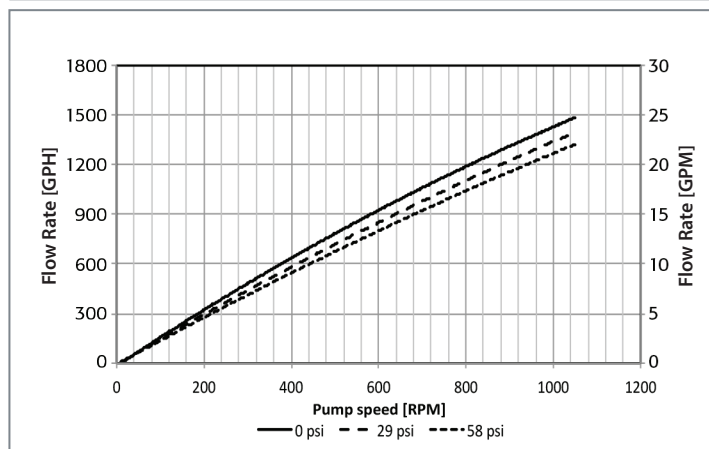
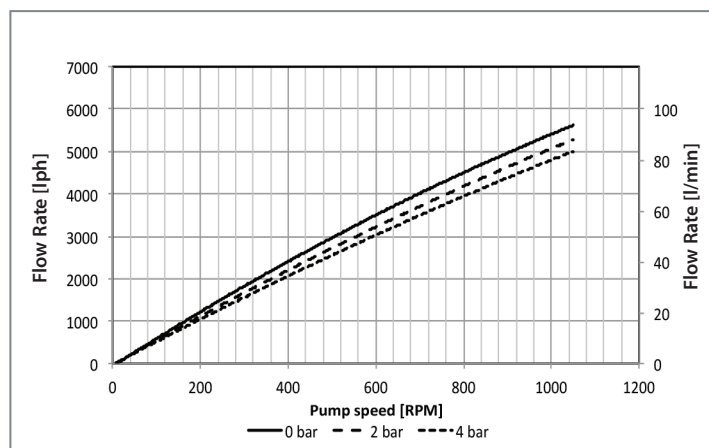
QF5050SU Servo Motor		
Flow Rate Maximum:	Eccentric Shaft 6°	5000 lph (83 lpm)
Flow Rate Minimum:	Eccentric Shaft 6°	50 lph (0.83 lpm)
Pressure:	Temperature of Fluid < 40° C (104° F)	4 bar (58 psi)
	Temperature of Fluid > 40° C (104° F)	4 bar (58 psi)
Maximum Temperature:	Fluid	60° C (140° F)
	Autoclave	130° C (266° F)
Suction Lift Dry at 1800 rpm:	Eccentric Shaft 6°	4 - 4.5 m (13.1-14.7 ft)
Volume Specifications:	Approximated Volume per Revolution at Free Output	95 ml
	Filling Volume Without Connectors	820 ml
Connection Specification (Standard):	Connectors	1.5" TC
	Position of Connectors	Front
Product Wetted Materials (Standard):	Pump Chamber	PP
	Valve Plate	PP
	Diaphragms	TPE
	Valves	EPDM / SS316L
	O-rings	EPDM
Certificates/Proofs (Optional):	Elastomere (product wetted)	USP <88> Cl. VI; FDA21CFR177; BSE/TSE Safe; USP 87/381/661
	Rated speed	3000 min <sup>-1</sup> (2.66:1 reduction)
Motor:	Voltage	400 V
	Power	3 KW
Pump Dimension with Motor and Housing:	Length	440 mm (17.32")
	Width	325 mm (12.80")
	Height	320 mm (12.60")
Pump Weight with Motor and Housing:		51 kg (112 lb.)

Other connection specifications, materials and motors available on request.



### Performance Charts

#### Eccentric Shaft: 6°



Depending on the selected motor/frequency drive combination, the motor frequency and the resulting pump speed might differ.

## Accessories



### Control Box

- Variable speed controller with integrated key pad for manual speed control
- Configurable for remote speed control with 4 – 20 mA analogue input
- 230V / 50 Hz or 115 V / 60 Hz for model 1200 (image left)
- 400V, 3P for models 4400/5050/20k (image right)
- Hygienic 1.4301 housing, IP 54
- Easy plug & play installation

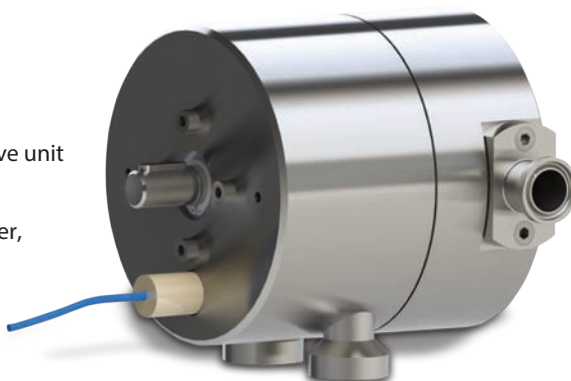


### Power Box

- Plug & Play installation
- Protects system and pump from overpressure
- Configurable pressure switch setpoint
- Reset button for pump reset
- To be used with pressure switch (also available)
- For multiple-use models only

### Diaphragm Sensor

- Sensor installed in ring drive unit
- Detection of all liquids
- Signal output to a controller, if diaphragm is ruptured



### PID Pressure Controller

- Ideal for processes, where the Quattroflow pump should be controlled to a defined pressure or flow rate (e.g. for filtration)
- 4 – 20mA input for pressure or flow sensor
- 24VDC voltage supply for sensors
- Autotune function for optimization of PID parameters
- 0 – 5VDC output signal for use with QF150 or QF1200CV (requires optional analogue input)
- Configurable alarm setpoints for automatic shutoff of pump







# Concerned about Particle Generation?

## Quattroflow™ pumps and peristaltic pumps: Particle generation compared

Facts about peristaltic pumps used for biopharmaceutical production processes:

- Particle generation caused by pump design.
- Permanent mechanical stress of the hose may lead to a substantial source of particles entering the fluid stream.
- Possible contamination of the pumped liquid and the pharmaceutical end product.
- Drop-in flow rate over time
- Some are not capable of reaching more than 1 bar

### Do you want to avoid this in your product?

The images on the right show micrographs of filter membranes, which were used to quantify particles created during a pumping process.

- Particles from the peristaltic pump are visible as bright objects obstructing the pores of the membrane (*lower image*).
- The filter membrane of the Quattroflow test does not show particles just open membrane pores. The gentle working principle of the 4-piston Quattroflow pump minimizes mechanical stress and thus the generation of particles (*upper image*).

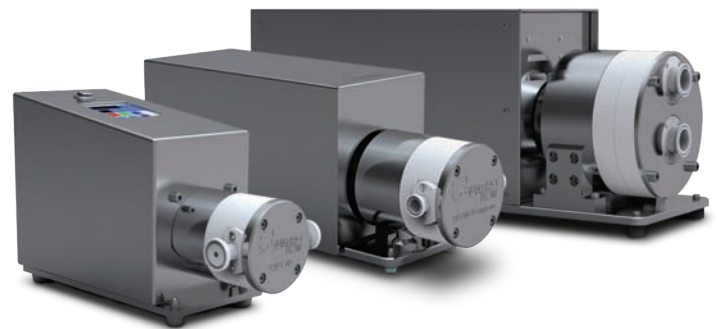
#### Test conditions:

Third party comparison between Quattroflow QF150SU and peristaltic pump using pharma-grade pumping hose. 8h continuous recirculation through 12µm filter filter at approximately 100 lph (1.67 lpm)

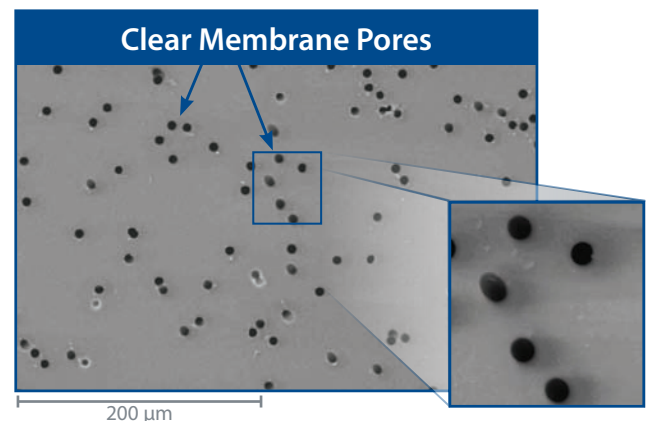
#### Result:

- 2 Mio particles with sizes between 6.1 and 12.7 µm for the peristaltic pump.
- No particles identifiable for the Quattroflow pump.

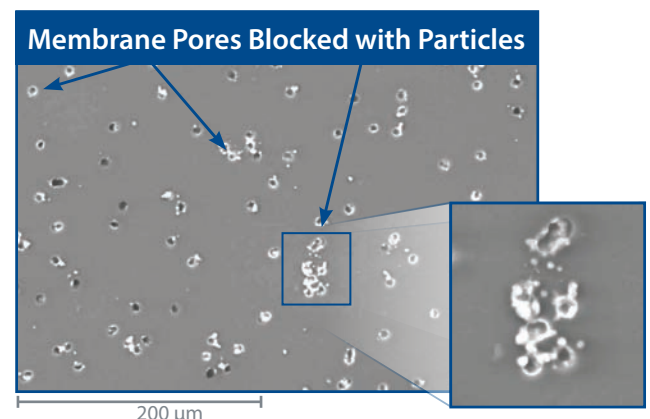
Quattroflow pumps help minimize particle contamination of your product, reduce heat, and are suitable for securing the handling of expensive and/or sensitive liquids.



#### Quattroflow



#### Peristaltic Pump





# Quattroflow™ Pumps make the difference

Besides the particle generation and product contamination facts mentioned on the first page, peristaltic pumps have some further operational limitations, which can be a disadvantage and risk for your process:

## **Pulsation**

Due to their operational design, peristaltic pumps create a pulsing flow, which can adversely affect the process.

## **Tube failure**

High mechanical stress can result in tube rupture, which can lead to a catastrophic failure, costly product loss, downtime and maintenance.

## **Flow rate consistency**

With increasing operating time of the tube, mechanical stress changes the hose geometries over time and can lead to an inconsistent flow.

These disadvantages inherent in peristaltic pumps ultimately mean potential threats to the quality of the process and the final product. Spallation, performance loss and rupture are also described in scientific literature (see Bahal and Romansky, "Spalling and sorption of tubing for peristaltic pumps") in *Pharmaceutical Development and Technology*, 7(3), 317-323 (2002).

Single-use technologies have created improved production opportunities in bio-pharmaceutical production processes. The correct pump technology can make a significant contribution.

## **Particle generation outside the hose**

Spallation release may also occur outside the hose. This may compromise the fluid path but also contaminate the external clean room environment.

## **Pump technology change**

Limited flow and pressure capabilities of peristaltic pumps means changing pump technologies as processes move from process development to cGMP creating scale up issues.





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## Where Innovation Flows



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Authorized PSG Partner:



250 mL Blood Bag System  
CPDA-1 Anticoagulant



## TERUFLEX® 250mL Blood Bag System with 16 Gauge Needle

- **For whole blood collection:**

From pediatric, elderly or small autologous donors who may not tolerate larger blood collections

As directed by a patient's physician

- **Red cell storage up to 35 days**

- **Tamper Evident Needle Protector**

The black lines of the needle protector and hub are conveniently aligned to:

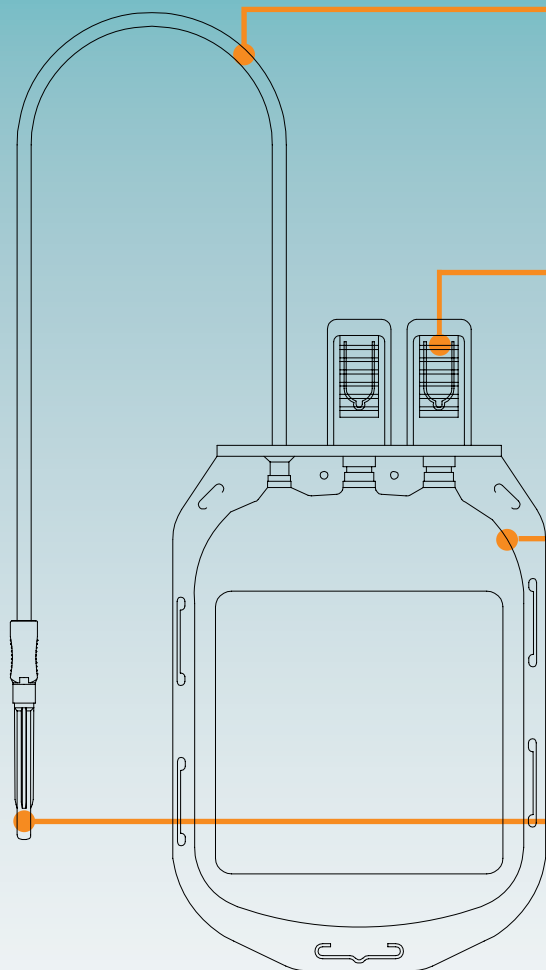
Show evidence of a broken seal

Indicate bevel up position

**TERUFLEX® blood bags and accessories contain no latex components**

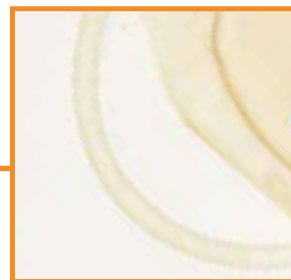


# Flexibility and Performance



- **Thick Wall Tubing**

Reduces kinks which facilitates smooth blood flow



- **Outlet Port Peel Tab**

Easy to open  
Visual assurance of seal integrity



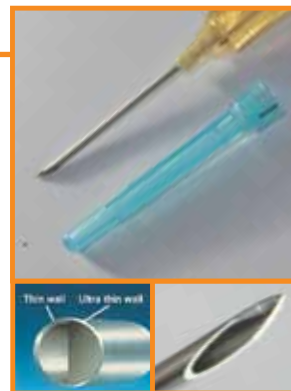
- **Bag Design**

Tall narrow design enhances blood component separation  
Rounded corners facilitate mixing of blood and anticoagulant



- **Sharp, Dependable Needle**

Double Bevel Design  
Ultra-thin Wall Needle



**TERUMO MEDICAL CORPORATION**

2101 Cottontail Lane Somerset,  
New Jersey 08873  
Tel: 800.283.7866

## Ordering Information:

Product Code: 1BB\*SCD256A

Quantity: 90 blood bags/case

Shelf Life: 30 months

Contains no latex components



# Single-Use BioProcess Containers

Standard and customized 2D and 3D  
fluid containment systems

# Single-use technologies

Whether you are looking for an economical catalog product or require a specific custom-designed and custom-built system, we offer proven solutions at every scale. With a wealth of experience, knowledge, and solid quality processes behind every Thermo Scientific™ product, we will help you feel assured that your bioproduction process performs at its best.



We take pride in offering one of the largest libraries and catalog components in the industry, allowing us to integrate connectors, tubing, and sensors to design a custom Thermo Scientific™ BioProcess Container (BPC) or a customized tubing assembly to fit your specific needs. Our services organization is here to assist you with drawings, implementation, and technical support to help ensure optimum production performance.

Our products and services are designed to enable proven performance through innovative and highly effective upstream or downstream applications. You can optimize production, improve process efficiency, add flexibility, and ensure fast-track product development and introduction to market by selecting product components and services from a single supplier.

## **Quality control and assurance**

To help ensure that BPC systems conform to the quality standards expected in the bioprocess industry, BPC systems are subject to rigorous quality control in compliance with current good manufacturing practices (cGMP) (21 CFR Part 820) and ISO 9001:2000 from the receipt of components to the release of final product.

Our production control processes help ensure complete lot traceability for each batch. The process control document becomes the stepwise manufacturing record that physically accompanies the lot through every step of the manufacturing process. At the end of the process, the production record is reviewed by the quality assurance team for completeness, and correctness prior to the release of the lot and issuance of the certificate of analysis (CoA).



# What is a Single-Use BioProcess Container?

The heart of our single-use solutions is our BioProcess Containers (BPCs). BPCs are single-use, flexible container systems commonly used for critical liquid-handling applications in the biopharmaceutical industry.

BPC systems are cost-effective alternatives to conventional stainless steel systems. They employ a novel design approach that is highly valued for its versatility and utility. BPC components are readily integrated into a variety of high-performance systems for all steps in the production of biologics.

## Key features

- All BPCs are produced in state-of-the-art cGMP facilities with common processes for manufacturing redundancy
- Production of chambers from 50 mL to 10,000 L capacity
- Automated lines for producing BPC chambers
- Strong engineering support to design and maintain products and processes

## BPC manufacturing process

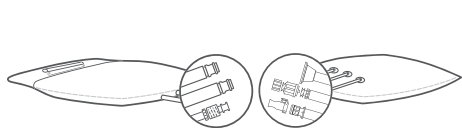
**Chamber manufacture**—the main components of a BPC chamber are the ports that enable tubing to be attached to the chamber. There are a number of different port designs depending on the type of chamber.

All BPC chambers and related components are produced in an ISO 7–certified cleanroom at our manufacturing facilities. Additional components are then attached to the BPC chamber to produce a complete BPC. BPC assembly is a manual process, which provides the required flexibility in BPC configuration. Thermo Scientific™ fluid transfer assemblies are also produced to complement BPC systems. Final assembly is done under the same controlled environment and to the same level of quality.

Each lot of BPCs is 100% visually inspected against product specifications, and packaged and sealed in two independent outer layers while still in the ISO 7–certified area. They are then placed in cardboard cartons labeled with product and lot identification.

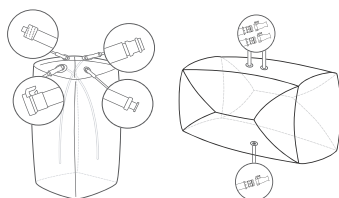
## Main types of BPC chambers

The 3 main types of BPCs are highlighted below, which are Thermo Scientific™ 2D Labtainer™ and 3D Productainer™ BPCs, and tank liners. Specialty BPCs are also available for specific applications and use in bioprocess equipment.



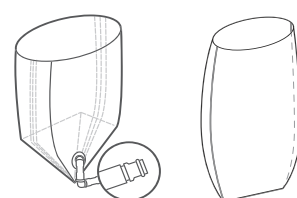
### 2D Labtainer BPC systems

This design is used for small, simple BPCs and is produced from two sheets of film that are heat-sealed around the perimeter to form a pillow-shaped chamber. The ports are heat-sealed into the end seal or onto one of the faces of the chamber.



### 3D Productainer BPC systems

This design is used for larger and more complicated BPCs. A square tube is formed by heat-sealing sheets of film together. Top- and bottom-porting options are available, and a greater range of size and complexity of chamber designs is possible.



### Tank Liner BPC systems

This design is used with commercially available overhead mixers. Tank liners remove the need for tank cleaning and helps reduce cycle times. Tank liners are optimized for use with Thermo Scientific™ drums and commonly used industry-standard cylindrical tanks.



# BPC films

## Films engineered for the most demanding applications

The Thermo Scientific™ films are engineered to meet the most demanding requirements of your bioproduction processes. Choose between:

- **Aegis™ 5-14 film**—for high-value applications
- **CX5-14 film**—one of the most widely used films in the industry, proven over 10 years
- **ASI™ 26/77 film**—a two-layered film, for high-value applications
- **ASI™ 28 film**—a robust, four-layer film with excellent oxygen- and moisture-barrier properties
- **CX3-9 film**—a three-layered film with a polyester elastomer coextruded outer layer with a low-density polyethylene product contact layer
- **ASI™ 26 film**—a single-web film with good toughness and puncture resistance
- **ASI™ 77 film**—a single-web film constructed from nylon with an ethylene vinyl alcohol EVOH barrier layer and a linear low-density polyethylene (LLDPE) layer

## Film formulations

- Aegis5-14 and CX5-14 films are five-layer films produced in a cGMP facility—the outer layer is a polyester elastomer coextruded with an EVOH barrier layer and a low-density polyethylene product contact layer
- The ASI 26/77 polyethylene (PE) single-use bag systems are produced in a cGMP facility, utilizing two webs: a fluid contact film and a durable, coextruded outer-layer film
- The ASI 28 film is an ethylene vinyl acetate (EVA) coextruded film produced in a cGMP facility—this film provides an excellent, highly durable moisture and oxygen barrier
- The CX3-9 film is a three-layer, 9 mil cast film, which is used primarily for open-top tank liners
- The ASI 26 film is a single-web, 5 mil cast film, which is also used primarily for open-top tank liners
- The ASI 77 film is a single-web, 7 mil cast film, which is used primarily for the Thermo Scientific™ Three60™ Sampling System





## 2D Labtainer BPC systems

### Key features

- 2D Labtainer systems are 2-panel, pillow-style BPCs
- Chambers are constructed from CX5-14, Aegis5-14, ASI 26/77, and ASI 28 films
- Labtainer BPC systems have 2–3 edge ports along one end with a handle on the opposing end
- 2-port Labtainer BPCs are available in sizes from 50 mL to 2,000 mL
- 3-port Labtainer BPCs are available in sizes from 2 to 50 L
- Line sets can be customized for easy integration with existing process operations and equipment
- Labtainers can be customized into multi-container manifold configurations
- Labtainers can be added as sample container adjuncts to 2D or 3D BPCs

### Applications

- Harvest from bioreactors or fermentors
- Feed into bioreactors or fermentors
- Sample collection from bioreactors or fermentors
- Buffer preparation and storage
- Culture media preparation and storage
- Process liquid preparation and storage
- Chromatography feed and fraction collection
- Harvest, storage, and transport of bulk drug product and bulk drug precursors

# 3D Productainer BPC systems

## Key features

- Use this system to eliminate post-use cleaning steps required with reusable containers, and to reduce cross-contamination risks
- All 3D BPCs are constructed in an ISO 7–certified cleanroom under cGMP conditions
- All 3D BPCs are designed to fit the full range of support containers, both square and cylindrical, from 50 to 3,000 L

## Applications

- Hydration and filtration of process buffers, liquids, and culture media
- Chromatography feed and fraction collection
- Storage and transport of bulk drug product and bulk drug precursors
- Harvest from and feed into bioreactors and fermentors
- Dispensing, packaging, and storage of cell culture media, buffers, and process liquids

## Customize catalog BPC systems for optimized single-use technology

Choose from the industry's largest component library—with over 2,000 unique components that include:

- **Fittings**—straight, reducing, elbow, T-style, X-style, and cross-style
- **Connectors**—quick connectors and disconnectors, aseptic connectors and disconnectors, steam-to and steam-through connectors
- **Filters**—used for bioburden reduction; sterilization-grade filters and vent filters are available
- **Tubing**—thermoplastic elastomers, platinum-cured silicone
- **Sample ports**—septum-style and needle-free ports
- **Clamps**—sliders, pinch, and crimp bands







## Tank liner BPC systems

### Key features

- Tank liners are designed for use with commercially available overhead mixers (not supplied)
- Removes the need for tank cleaning and helps reduce cycle times.
- Chambers are constructed from CX3-9 and ASI 26 films (and Aegis5-14 film for bottom-drain) with dimensions optimized for Thermo Scientific™ catalog drums and commonly used industry-standard cylindrical tanks
- Top entry for catalog products for maximum recovery using industry-standard cylindrical tanks in unit volumes of 50, 100, 200, 300, and 500 L
- Supplied gamma-irradiated to minimize bioburden

### Associated applications

- Hydration of powdered media and buffers
- Pooling of nonsterile solutions and fluids





# Powdertainer BPC system

Thermo Scientific™ Powdertainer™ BPCs are specifically designed for powder containment and discharge applications, and maintain a closed system for maximum recovery of powder while minimizing the risk of cross-contamination.

## Key features

- Designed for powder containment and powder hydration applications
- Employ a closed system to help minimize dust contaminant and cross-contamination risk
- Three-inch tri-clamp port designed for secure connection to, and easy integration with, hydration vessels
- Suspensor handle for support during discharge and neck clamp to retain powder prior to discharge
- Two models, including one with a washdown line to remove residual powder, enabling maximal recovery
- Three sizes for process flexibility: 1, 5, and 25 kg
- Constructed from CX3-9 film
- Stainless steel filling stand to facilitate the powder discharge process

## Applications

- Storage and delivery of Thermo Scientific™ powder culture media and buffers
- Storage and dispensing of preweighed chemicals or other process powders

### Handle

For support during emptying

### Tube with quick connector

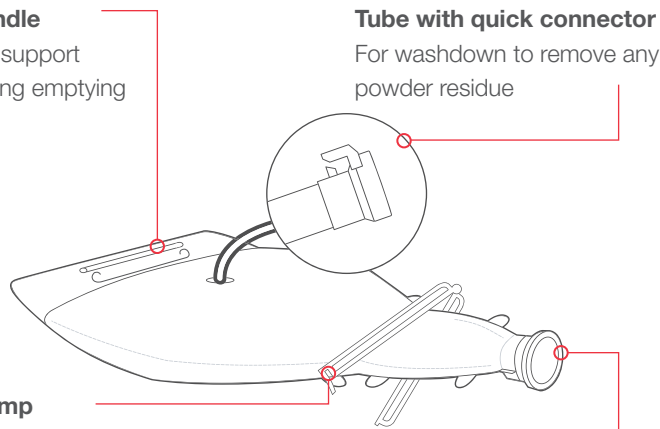
For washdown to remove any powder residue

### Clamp

To retain powder prior to emptying

### Three-inch tri-clamp connector

For secure connection to formulation vessel



# Harvestainer Microcarrier Separation BPC system

The Thermo Scientific™ Harvestainer™ BioProcess Container (BPC) system is a closed, single-use microcarrier separation system that helps to increase product yields compared to traditional methods, while reducing clean-in-place (CIP) and steam-in-place (SIP) requirements.

## Unique design features

The Harvestainer system enables separation of microcarrier beads and harvesting of the cell culture supernatant in a single-step, closed system.

The Harvestainer system is designed for both small- and large-scale applications. When 12 L or less of microcarrier beads are required to be separated, the 3 L or 12 L Harvestainer system is ideal. These systems are designed around our 2D pillow-style BPC in a preassembled tray, designed for secondary containment and optimal supernatant recovery.



The large-scale Harvestainer system features a dual-chamber system that comprises a 200 L 3D BPC with either one or two interior 25 L microcarrier 2D BPCs. These unique design features help enable the separation of cell culture supernatant from the microcarrier beads.





# Three60 Single-Use Sampling System

The simple design behind the Three60 Sampling System allows you to take a representative sample of your product with minimal effort. For a small-volume liquid transfer, use a CIP or SIP process to prepare your tank. The pre-irradiated BPCs and assemblies help ensure an integral fluid path while the quick-turn valve and pinch-and-cut disconnectors maintain liquid transfer and removal from BPCs.

The Three60 system is compatible with your vessel through a sanitary connector; no expensive hardware is needed. Each Three60 package contains a valve and four assemblies with pinch-and-cut disconnectors.

## Kit to tank

The Three60 system is pre-irradiated and assembled into a one-piece kit. Simply remove the device from the kit and apply to the vessel.

- **Quick-turn Three60 valve**—the face of the Three60 valve can be sterilized with the tank through traditional CIP/SIP processes. The valve has four assemblies. This helps keep the product and technician contamination-free.

- **Pinch-and-cut disconnectors**—the pinch-and-cut disconnectors allow the technician to quickly separate the sample and eliminate the need for tools or tube sealing.
- **Injection ports**—Luer lock injection site; extract through either the septum or twist-off Luer lock to pour.
- **Pre-irradiated BPC assemblies**—the Three60 system is provided with pre-irradiated BPC assemblies in sizes ranging from 50 mL to 2 L produced using the ASI 77 film.



# Services and support

Thermo Scientific™ Bioproduction services involve both field- and office-based teams. The office-based team is located in Logan, Utah, and includes technical support, BioProduction Xpress (BPX), quality, and services management teams. On a global scale, there are field application specialists (FAS) who are trained on hardware installation, servicing, and also offer other post-sales support such as training, consulting, and process optimization assistance.

## Key services

### • Field support

- Hardware installation—factory acceptance test (FAT) and site acceptance test (SAT)
- Training—single-use bioreactors, mixers, fermentors, outer support containers, and BPCs
- Validation service—installation qualification (IQ), operation qualification (OQ), and performance qualification (PQ)
- Warranty
- Service contracts

### • BioProduction Xpress

- R&D scientists and engineers specializing in single-use bioreactor (S.U.B.), single-use mixer (S.U.M.), single-use fermentor (S.U.F.) processes
- Scale-up studies
- Customer-specific application studies
- Quality testing
- Non-GMP quick-response studies or tests

### • Technical support

- Phone support for sales, service, technical questions, and troubleshooting
- Interfaces with quality, product management, and R&D teams





## Integrated solutions for bioproduction

### Single-Use Mixers (S.U.M.s)

A variety of options up to 5,000 L for both upstream and downstream applications



### BioProcess Containers (BPCs)

A variety of configurations up to 2,000 L for liquid harvest, storage, and transportation



### Single-Use Bioreactors (S.U.B.s)

50–2,000 L bioreactors capable of integrating with an existing control system



### Liquid- and dry-format media

We offer both custom manufacturing and a full range of chemically defined performance media and supplement products



### Sera

Our sera are well known for consistent quality and reliability



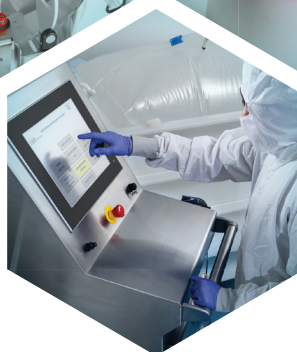
### Buffers and process liquids

Custom and standard buffers, and process liquids, including Gibco™ Water for Injection (WFI)



### Integrity testing systems

A true point-of-use integrity testing system to confirm the integrity of BPCs before use



Find out more at [thermofisher.com/bpc](https://thermofisher.com/bpc)

**ThermoFisher**  
SCIENTIFIC

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## Product datasheet

# Anti-Blood Group A Antigen antibody [9A] ab20131

## 2 References

### Overview

<b>Product name</b>	Anti-Blood Group A Antigen antibody [9A]
<b>Description</b>	Mouse monoclonal [9A] to Blood Group A Antigen
<b>Host species</b>	Mouse
<b>Specificity</b>	ab20131 cross reacts with EGF receptor in A431 cells only due to glycosylation defects in these cells which reveals otherwise cryptic sugar structures.
<b>Tested applications</b>	<b>Suitable for:</b> ELISA, IP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Cell preparation: A431 cells (human vulval squamous carcinoma cell line).

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C or -80°C. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	Preservative: None Constituents: PBS
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	9A
<b>Myeloma</b>	P3-x63-Ag8
<b>Isotype</b>	IgG3
<b>Light chain type</b>	unknown

### Applications

Our [Abpromise guarantee](#) covers the use of **ab20131** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ELISA		Use at an assay dependent dilution.

Application	Abreviews	Notes
IP		Use at an assay dependent dilution.

## Target

### Relevance

Blood-group antigens are generally defined as molecules formed by sequential addition of saccharides to the carbohydrate side chains of lipids and proteins detected on erythrocytes and certain epithelial cells. The A, B and H antigens are reported to undergo modulation during malignant cellular transformation. Blood group related antigens represent a group of carbohydrate determinants carried on both glycolipids and glycoproteins. They are usually mucin-type, and are detected on erythrocytes, certain epithelial cells, and in secretions of certain individuals. Sixteen genetically and biosynthetically distinct but inter-related specificities belong to this group of antigens, including A, B, H, Lewis A, Lewis B, Lewis X, Lewis Y, and precursor type 1 chain antigens.

### Cellular localization

Cell Membrane

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

## Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <http://www.abcam.com/abpromise> or contact our technical team.

## Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors

## SECTION 1: Identification of the substance/mixture and of the company/undertaking

### Identification of the substance or mixture

**Product code** 89964  
**Product name** HisPur(tm) Cobalt Resin, 10 mL

### Company/undertaking identification

Life Technologies Corporation  
5781 Van Allen Way  
PO Box 6482  
Carlsbad, CA 92008  
+1 760 603 7200

Life Technologies  
5250 Mainway Drive  
Burlington, ONT  
CANADA L7L 6A4  
800/263-6236

Thermo Fisher Scientific  
Pierce Biotechnology  
P.O. Box 117  
Rockford, IL 61105  
United States  
1.815.968.0747 or  
1.800.874.3723

### 24 hour Emergency Response:

866-536-0631  
301-431-8585  
Outside of the U.S. +1-301-431-8585

### Country specific Emergency Number (if available):

CHEMTREC Brazil (Rio De Janeiro) +(55)-2139581449 (português)

Use as laboratory reagent. Scientific research and development.

## SECTION 2: Hazards identification

### GHS - Classification

#### Signal Word

DANGER



### Health hazards

Carcinogenicity	Category 1A
Reproductive Toxicity	Category 1B

**Revision date** 15-Mar-2016  
**Product code** 89964

**Page** 1 / 6  
**Product name** HisPur(tm) Cobalt Resin, 10 mL



**Physical hazards**

GHS Physical Hazard 1	Flammable liquids
GHS Physical Hazard Category Number	Category 3

**Hazard Statements**

H226 - Flammable liquid and vapor

H350 - May cause cancer

H360F - May damage fertility

H411 - Toxic to aquatic life with long lasting effects

**Precautionary Statements**

P370 + P378 - In case of fire, use water/water spray/water jet/carbon dioxide/sand/foam/alcohol resistant foam/chemical powder for extinction

P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower

P201 - Obtain special instructions before use

P273 - Avoid release to the environment

P210 - Keep away from heat/sparks/open flames/hot surfaces. - No smoking

P280 - Wear protective gloves/protective clothing/eye protection/face protection

**Principle Routes of Exposure****Potential Health Effects**

**eyes**

May cause eye irritation with susceptible persons.

**Skin**

May be harmful in contact with skin.

**inhalation**

May cause irritation of respiratory tract.

**Ingestion**

May be harmful if swallowed.

**Specific effects**

**Carcinogenic effects**

May cause cancer.

**Mutagenic effects**

None.

**Reproductive toxicity**

May cause harm to the unborn child.

**Sensitization**

None.

**Target Organ Effects**

None under normal use conditions.

**HMIS**

Health	4 * Chronic Hazard
Flammability	3
Reactivity	0

**SECTION 3: Composition/information on ingredients**

Component	CAS-No	EINECS-No	Weight %
Cobalt(II) chloride hexahydrate 7791-13-1 ( 0.5-1 )	7791-13-1	-	0.5-1

We recommend handling all chemicals with caution.

**SECTION 4: First aid measures**

Revision date 15-Mar-2016  
Product code 89964

Page 2 / 6  
Product name HisPur(tm) Cobalt Resin, 10 mL

**Skin contact** Wash off immediately with plenty of water for at least 15 minutes. Take off all contaminated clothing and wash it before reuse. Immediate medical attention is required.

**Eye contact** Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.

**Ingestion** Call a physician or poison control center immediately. Never give anything by mouth to an unconscious person. Do not induce vomiting without medical advice.

**Inhalation** Remove to fresh air. If not breathing, give artificial respiration. Call a physician or poison control center immediately.

**Most important symptoms and effects, both acute and delayed**  
H226 - Flammable liquid and vapor, H350 - May cause cancer, H360F - May damage fertility, H411 - Toxic to aquatic life with long lasting effects

**Notes to Physician** Treat symptomatically.

## SECTION 5: Firefighting measures

**Suitable extinguishing media** Water spray. Carbon dioxide (CO<sub>2</sub>). Foam. Dry chemical.

**Special protective equipment for firefighters** Wear self-contained breathing apparatus and protective suit.

**Specific hazards arising from the chemical** Not known

## SECTION 6: Accidental release measures

**Personal precautions** Ensure adequate ventilation. Avoid contact with skin, eyes or clothing. Use personal protection equipment.

**Methods for cleaning up** Soak up with inert absorbent material.

### Environmental precautions

Prevent further leakage or spillage if safe to do so. Prevent product from entering drains.

See Section 12 for more information.

## SECTION 7: Handling and storage

**Handling** Always wear recommended Personal Protective Equipment. No special handling advices are necessary.

**Storage** Keep in a dry, cool and well-ventilated place. Keep in properly labeled containers.

## SECTION 8: Exposure controls/personal protection

### Exposure Limits

Chemical Name	OSHA PEL	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Cobalt(II) chloride hexahydrate	None	None	0.02 mg/m <sup>3</sup>	None

**Engineering measures** Ensure adequate ventilation, especially in confined areas.

### Personal Protective Equipment

Personal Protective Equipment requirements are dependent on the user institution's risk assessment and are specific to the risk assessment for each laboratory where this material may be used.

**Respiratory protection** In case of insufficient ventilation, wear suitable respiratory equipment.

**Hand protection**  
**Eye protection**  
**Skin and Body Protection**  
**Hygiene measures**

Impervious gloves.  
Wear safety glasses with side shields (or goggles).  
Lightweight protective clothing.  
Handle in accordance with good industrial hygiene and safety practice.

**Environmental exposure controls**

Prevent product from entering drains. Should not be released into the environment.

## SECTION 9: Physical and chemical properties

### **General information**

<b>Form</b>	Liquid	
<b>Appearance</b>	No information available	
<b>Odor</b>	Mixture has not been tested	
<b>Odor Threshold</b>	Mixture has not been tested	
<b>Boiling point / boiling range</b>	°C No data available	°F No data available
<b>Melting point / melting range</b>	°C No data available	°F No data available
<b>flash point</b>	°C 32.222	°F 89.996
<b>Autoignition Temperature</b>	°C No data available	°F No data available
<b>Evaporation rate</b>	No data available	
<b>Flammability (solid, gas)</b>	No data available	
<b>Oxidizing properties</b>	Mixture has not been tested	
<b>Water solubility</b>	No data available	
<b>Upper explosion limit</b>	No data available	
<b>Lower explosion limit</b>	No data available	
<b>Partition coefficient: n-octanol/water</b>	No data available	
<b>Vapor Pressure</b>	No data available	
<b>vapor density</b>	No data available	
<b>Viscosity</b>	Mixture has not been tested	
<b>pH value</b>	No data available	

## SECTION 10: Stability and reactivity

<b>Stability</b>	Stable under normal conditions.
<b>Materials to avoid</b>	No information available.
<b>Possibility of hazardous reactions</b>	Hazardous reaction has not been reported
<b>Hazardous decomposition products</b>	No information available.
<b>polymerization</b>	None under normal processing.
<b>Conditions to avoid</b>	None under normal processing.

## SECTION 11: Toxicological information

### **Acute Toxicity**

Revision date 15-Mar-2016  
Product code 89964

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Product name HisPur(tm) Cobalt Resin, 10 mL

Chemical Name	LD50 (oral, rat/mouse)	LD50 (dermal, rat/rabbit)	LC50 (inhalation, rat/mouse)
Cobalt(II) chloride hexahydrate	= 766 mg/kg (Rat) = 80 mg/kg (Rat)	No data available	No data available

## Principle Routes of Exposure

### Potential Health Effects

<b>eyes</b>	May cause eye irritation with susceptible persons.
<b>Skin</b>	May be harmful in contact with skin.
<b>inhalation</b>	May cause irritation of respiratory tract.
<b>Ingestion</b>	May be harmful if swallowed.
<b>Carcinogenic effects</b>	May cause cancer.
<b>Mutagenic effects</b>	None.
<b>Reproductive toxicity</b>	May cause harm to the unborn child.
<b>Sensitization</b>	None.
<b>Target Organ Effects</b>	None under normal use conditions

## SECTION 12: Ecological information

<b>Ecotoxicity</b>	Hazardous to the Aquatic Environment.
<b>Chronic aquatic toxicity</b>	Category 2.
<b>Mobility</b>	No information available.
<b>Biodegradation</b>	No information available.
<b>Bioaccumulation</b>	No information available.

## SECTION 13: Disposal considerations

Dispose of contents/containers in accordance with local regulations.

## SECTION 14: Transport information

### IATA

<b>Proper Shipping Name</b>	Flammable liquids, n.o.s. (ethanol, mixture)
<b>Hazard Class</b>	3
<b>Subsidiary class</b>	None
<b>Packing group</b>	III
<b>UN-No</b>	UN1993
<b>Environmental hazards</b>	No

## SECTION 15: Regulatory information

Component	US TSCA
Cobalt(II) chloride hexahydrate 7791-13-1 ( 0.5-1 )	-

### US Federal Regulations

#### **SARA 313**

This product contains the following toxic chemical(s) subject to the notification requirements of section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986. This law requires certain manufacturers to report on annual emissions of specified chemicals and chemical categories. Please note that if you repackage, or otherwise redistribute, this product to industrial customers, a notice similar to this one should be sent to those customers:.

<u>Chemical Name</u>	<u>CAS-No</u>	<u>Weight %</u>	<u>SARA 313 - Threshold Values</u>
Cobalt(II) chloride hexahydrate	7791-13-1	0.5-1	0.1

#### **Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)**

This product contains the following HAPs:.

<u>Component</u>	<u>CAS-No</u>	<u>Weight %</u>	<u>HAPS data</u>
Cobalt(II) chloride hexahydrate 7791-13-1 ( 0.5-1 )	7791-13-1	0.5-1	Present (including any unique chemical substance that contains Cobalt as part of its infrastructure)

### US State Regulations

#### **California Proposition 65**

This product does not contain any Proposition 65 chemicals.

#### **WHMIS Hazard Class**

B3 - Combustible liquid

D2A - Very toxic materials



This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

## SECTION 16: Other information

#### **Reason for revision**

SDS sections updated.

Use as laboratory reagent. Scientific research and development.

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRENTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE"

**End of Safety Data Sheet**

Revision date 15-Mar-2016  
Product code 89964

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Product name HisPur(tm) Cobalt Resin, 10 mL

# HisPur™ Cobalt Resin

**89964 89965 89966**

1851.2

Number	Description
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89964	HisPur Cobalt Resin, 10mL settled resin
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89965	HisPur Cobalt Resin, 100mL settled resin
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89966	HisPur Cobalt Resin, 500mL settled resin
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Binding Capacity:  $\geq 10\text{mg}$  at  $> 90\%$  purity of a 28kDa His-tagged protein from a bacterial source per milliliter of settled resin

Resin: Crosslinked 6% agarose in a 20% ethanol solution

**Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

## Introduction

The Thermo Scientific HisPur Cobalt Resin enables efficient purification of polyhistidine-tagged proteins from bacterial, mammalian and baculovirus-infected cells. His-tagged proteins are purified from total soluble protein extract using a cobalt-charged tetradentate chelator immobilized onto 6% crosslinked agarose. The cobalt resin is compatible using native or denaturing conditions and can be used in multiple formats including conventional gravity flow chromatography, spin column and FPLC.

Many immobilized metal affinity chromatography (IMAC) resins use nickel ( $\text{Ni}^{+2}$ ) as the metal source for purifying His-tagged proteins. Although  $\text{Ni}^{+2}$  chelate resins achieve high protein yields, purity is often suboptimal, resulting in the need for additional optimization of wash and elution steps. Cobalt achieves both high protein yield and purity with minimal optimization. Furthermore, HisPur Cobalt Resin displays less metal leaching compared with  $\text{Ni}^{+2}$  resins.

## Important Product Information

- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein. Therefore, it is important to optimize these parameters before attempting a large-scale purification. For best results, perform a small-scale test to estimate the expression level and determine the solubility of each His-tagged protein.
- Optimization of the lysis procedure is critical for maximizing protein yield. Some methods for protein extraction include using commercially available detergent-based reagents, such as Thermo Scientific B-PER Bacterial Protein Extraction Reagent (Product No. 78248), and mechanical methods, such as freeze/thaw cycles, sonication or French press.
- Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8M urea, 6M guanidine or Inclusion Body Solubilization Reagent (Product No. 78115) and purified with the cobalt resin, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.
- One advantage of using cobalt is its low nonspecific binding. Although the buffer conditions described in these instructions work well for many samples, optimization may be required to further reduce nonspecific binding. To optimize conditions, adjust the imidazole concentration in the Equilibration/Wash Buffer or decrease the buffer's pH to protonate a greater proportion of histidine groups.
- IMAC relies on cobalt chelation to both the tetradentate chelator and the target histidine tag. Avoid using protease inhibitors or other additives that contain chelators, such as EDTA, or strong reducing agents, such as DTT or  $\beta$ -mercaptoethanol, which will disrupt the function of the cobalt resin.

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## Additional Materials Required

- MES Buffer: 20mM 2-(*N*-morpholine)-ethanesulfonic acid, 0.1M sodium chloride; pH 5.0

### For native conditions prepare the following buffers:

- Equilibration/Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 10mM imidazole; pH 7.4
- Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4

### For denaturing conditions prepare the following buffers:

- Equilibration/Wash Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 10mM imidazole; pH 7.4
- Elution Buffer: 50mM sodium phosphate, 300mM sodium chloride, 6M guanidine•HCl, 150mM imidazole; pH 7.4.

## Procedure for Purification of His-Tagged Proteins by Batch Method

The HisPur Cobalt Resin allows customization of a purification strategy. Purification conditions detailed within these instructions can be scaled as desired. The procedure may be performed at room temperature or at 4°C.

1. Add an appropriate amount of cobalt resin to a tube. Centrifuge tube for 2 minutes at  $700 \times g$  and carefully remove and discard the supernatant.
2. Add two resin-bed volumes of Equilibration/Wash Buffer and mix until the resin is fully suspended.
3. Centrifuge tube for 2 minutes at  $700 \times g$  and carefully remove and discard buffer.
4. Prepare sample by mixing the protein extract with an equal volume of Equilibration/Wash Buffer.
5. Add the prepared protein extract to the tube and mix on an end-over-end rotator for 30 minutes.
6. Centrifuge the tube for 2 minutes at  $700 \times g$ . If desired, save supernatant for downstream analysis.
7. Wash the resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge the tube for 2 minutes at  $700 \times g$ . If desired, save supernatant for downstream analysis.
8. Repeat wash step and monitor supernatant by measuring its absorbance at 280nm until baseline is reached.
9. Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer. Centrifuge tube for 2 minutes at  $700 \times g$ . Carefully remove and save the supernatant. Repeat this step twice, saving each supernatant fraction in a separate tube.
10. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay (Product No. 23236). The eluted protein can be directly analyzed by SDS-PAGE. To remove excess imidazole for downstream applications use gel filtration (e.g., Thermo Scientific Zeba Spin Desalting Columns) or dialysis (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes). Samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8 M urea before SDS-PAGE analysis. The Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) may also be used to remove guanidine.

## Procedure for Purification of His-tagged Proteins using a Gravity-flow Column

The HisPur Cobalt Resin allows customization of a purification strategy. Purification conditions detailed within these instructions can be scaled as desired. Perform the procedure at room temperature or at 4°C.

1. Pack column with an appropriate amount of cobalt resin. Allow storage buffer to drain from resin by gravity flow.
2. Prepare sample by mixing the protein extract with an equal volume of Equilibration/Wash Buffer.
3. Equilibrate column with two resin-bed volumes of Equilibration/Wash Buffer. Allow buffer to drain from resin, flow rate should be 0.5-1mL/minute.
4. Add the prepared protein extract onto the resin. Collect the flow-through in a tube. If desired, re-apply the flow-through once to maximize binding.
5. Wash resin with two resin-bed volumes of Equilibration/Wash Buffer and collect the flow-through. Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.

6. Elute His-tagged proteins from the resin with two resin-bed volumes of Elution Buffer. Repeat this step twice, collecting each fraction in a separate tube.
7. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay (Product No. 23236). The eluted protein can be directly analyzed by SDS-PAGE. To remove excess imidazole for downstream applications use gel filtration (e.g., Zeba™ Spin Desalting Columns) or dialysis (e.g., Slide-A-Lyzer® Dialysis Cassettes). Samples containing 6M guanidine•HCl must be dialyzed against buffer containing 8M urea before SDS-PAGE analysis. The Pierce® SDS-PAGE Sample Prep Kit (Product No. 89888) may also be used to remove guanidine.

## Procedure for Cobalt Resin Regeneration

The cobalt resin may be used up to three times without affecting protein yield or purity. Between each use, perform the procedure as described below to remove residual imidazole and any nonspecifically adsorbed protein. To prevent cross-contamination of samples, designate a given column to one specific fusion protein.

1. Wash resin with 10 resin-bed volumes of 20mM MES Buffer , 0.1M sodium chloride; pH 5.0.
2. Wash resin with 10 resin-bed volumes of ultrapure water.
3. Store resin as a 50% slurry in 20% ethanol.

## Troubleshooting

Problem	Possible Cause	Solution
Low protein yield	Poor expression of soluble protein	Optimize bacterial expression conditions
	His-tagged protein forms inclusion bodies	Alter bacterial growth conditions to minimize inclusion body formation and maximize soluble protein yield; alternatively, solubilize inclusion bodies and perform the purification with a compatible denaturant (e.g., Inclusion Body Solubilization Reagent, Product No. 78115)
	Insufficient cell lysis and extraction	Optimize cell lysis protocol
	Fusion protein does not bind to the column	Verify the sequence or perform an ELISA or Western blot using an antibody against the His tag to make sure the His-tag is present
Poor protein purity	Insufficient washing	Wash resin additional times or modify imidazole concentration and pH of the Equilibration/Wash Buffer
Slow column flow	Column is overloaded	Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate

## Additional Information

Please visit the website for additional information including the following items:

- Tech Tip #43: Protein stability and storage
- Tech Tip #40: Convert between times gravity (× g) and centrifuge rotor speed (RPM)
- Tech Tip #6: Extinction coefficients guide
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #13: Pack beaded affinity resin into affinity columns



## Related Thermo Scientific Products

16100	Pierce Glutathione Agarose, 10mL
88270	Pierce High Capacity Endotoxin Removal Resin, 10mL
88282	Pierce LAL Chromogenic Endotoxin Quantitation Kit
88221	HisPur Ni-NTA Resin, 10mL
89967	HisPur Cobalt Spin Columns, 0.2mL, 25 each
89968	HisPur Cobalt Spin Columns, 1.0mL, 5 each
89969	HisPur Cobalt Spin Columns, 3.0mL, 5 each
78248	B-PER <sup>®</sup> Bacterial Protein Extraction Reagent, 500mL
78260	B-PER II Bacterial Protein Extraction Reagent, 250mL
89802	I-PER <sup>®</sup> Insect Cell Protein Extraction Reagent, 250mL
78410	Halt <sup>™</sup> Protease Inhibitor Cocktail, EDTA-Free, 1mL
78115	Inclusion Body Solubilization Reagent, 100mL
89835	DNase I, 5000 units
23236	Coomassie Plus (Bradford) Assay
89890	Zeba Spin Desalting Columns, 7K MWCO, 2mL, 25/pkg
89892	Zeba Spin Desalting Columns, 7K MWCO, 5mL, 25/pkg
89894	Zeba Spin Desalting Columns, 7K MWCO, 10mL, 25/pkg
66385	Slide-A-Lyzer Dialysis Cassettes Kit, 10K MWCO, 0.1-0.5mL
66382	Slide-A-Lyzer Dialysis Cassettes Kit, 10K MWCO, 0.5-3mL
66807	Slide-A-Lyzer Dialysis Cassettes Kit, 10K MWCO, 3-12mL

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Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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# Pierce High-Capacity Endotoxin Removal Resin

MAN0016351

Rev A.0

Pub. Part No. 2162373.3

Number	Description
88270	<b>Pierce High-Capacity Endotoxin Removal Resin</b> , 10mL of settled resin supplied as 50% slurry in 20% ethanol
88271	<b>Pierce High-Capacity Endotoxin Removal Resin</b> , 100mL of settled resin supplied as 50% slurry in 20% ethanol
88272	<b>Pierce High-Capacity Endotoxin Removal Resin</b> , 250mL of settled resin supplied as 50% slurry in 20% ethanol
88273	<b>Pierce High-Capacity Endotoxin Removal Spin Column, 0.25mL</b> , 5 columns, each column contains 25% slurry in 20% ethanol
88274	<b>Pierce High-Capacity Endotoxin Removal Spin Column, 0.50mL</b> , 5 columns, each column contains 25% slurry in 20% ethanol
88275	<b>Pierce High-Capacity Endotoxin Removal Spin Column, 0.50mL</b> , 25 columns, each column contains 25% slurry in 20% ethanol
88276	<b>Pierce High-Capacity Endotoxin Removal Spin Column, 1.0mL</b> , 5 columns, each column contains 25% slurry in 20% ethanol
88277	<b>Pierce High-Capacity Endotoxin Removal Spin Column, 1.0mL</b> , 25 columns, each column contains 25% slurry in 20% ethanol

**Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

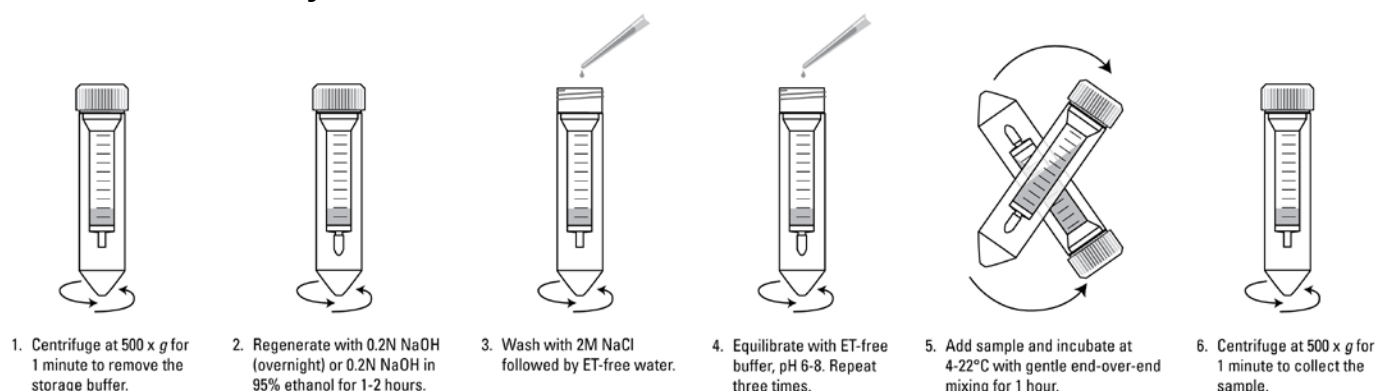
## Introduction

The Thermo Scientific™ Pierce™ High Capacity Endotoxin Removal Resin contains porous cellulose beads that have been surface modified with covalently attached, modified  $\epsilon$ -poly-L-lysine. Modified polylysine has a high affinity for endotoxins with the affinity ligand eliminating toxicity associated with alternative technologies using polymyxin B ligands and sodium deoxycholate buffers. The binding capacity of 2,000,000 Endotoxin Units (EU)/mL allows endotoxin levels to be reduced by 99% in samples containing 10,000EU/mL; typical protein samples processed with the resin have a final endotoxin concentration below 5EU/mL. The resin is offered in a slurry format for custom packing of endotoxin removal columns for gravity flow or continuous flow (10-15 mL/hour) applications or a spin column format for the fast, single-use batch method. Removal of endotoxins from differing volumes of biological samples can be done in  $\geq 1$  hour (see Table 1).

Endotoxins consist of lipopolysaccharides (LPS), which are biologically active, structural components of the outer cell membrane of all gram-negative bacteria. Small amounts of endotoxin in recombinant protein preparations can cause side effects, including endotoxin shock, tissue injury and death in host organisms; therefore, it is essential to remove endotoxins from drugs, injectables and other biological products.

One milliliter of this resin can reduce the endotoxin concentration from a 1mL sample containing 10,000EU to  $\leq 5$ EU.

## Procedure Summary



## Important Product Information

- All materials (e.g., containers, buffers and pipette tips) must be endotoxin-free. Use endotoxin-free water to prepare buffers and other solutions.
- Endotoxin binding to the resin occurs at pH 6-8; Equilibrate the resin with an endotoxin-free buffer at neutral pH that includes 10-50mM sodium phosphate buffer or Tris-HCl buffer containing 0.1-0.2M NaCl. Check the sample pH and adjust to pH 6-8 with 0.1M NaOH or 0.1M HCl.
- The speed of sample application and wash depends on the sample endotoxin state. Free endotoxin can bind quickly to the resin, but protein-bound endotoxin may require longer incubation, slower flow rates and/or multiple recycling of the sample through the column. Tightly bound endotoxin may be incubated overnight at room temperature or 4°C in batch mode with gentle shaking.
- Resins can be used a minimum of five times without loss of endotoxin-removal efficiency.
- Sample volumes vary depending on the chosen pre-loaded column size (see Table 1).

**Table 1. Sample volumes for the Thermo Scientific Pierce High-Capacity Endotoxin Removal Columns.**

<u>Column size (mL)</u>	<u>Sample volume (mL)</u>
0.25	0.5-1.0
0.50	1.0-4.0
1.0	2.0-10.0

## Additional Materials Required

- Endotoxin-free, ultrapure water
- Endotoxin-free 15mL conical collection tubes (for 0.25mL and 0.5mL spin columns)
- Endotoxin-free 50mL conical collection tubes (for 1.0mL spin columns)
- Regeneration buffer: 0.2N NaOH for overnight incubation at room temperature **OR** 0.2N NaOH and 95% ethanol for 1-2 hours of incubation at room temperature

**Note:** Regenerate the column before the first use and after each subsequent use.

- 2M NaCl solution: If possible, use ready-made endotoxin-free NaCl solution; alternatively, dissolve pure sodium chloride crystals in endotoxin-free water
- Variable-speed centrifuge with rotor and carriers capable of handling 15mL and 50mL conical collection tubes

## Endotoxin Removal Procedure using the Column Method

- Regenerate the resin before the first use and after each subsequent use.
  - Equilibrate all solutions and the resin to room temperature before use.
  - Degas the resin slurry before applying to the column to prevent air bubbles from clogging the column and reducing flow.
1. To degas the resin, place slurry in a suction filter flask and degas with gentle stirring.
  2. Pour the degassed resin slurry into an appropriately sized column and allow the resin to settle.
  3. Regenerate the resin by washing with five resin-bed volumes of 0.2N NaOH overnight at room temperature **OR** five resin-bed volumes of 0.2N NaOH in 95% ethanol for 1-2 hours at room temperature.
  4. Wash with five resin-bed volumes of 2M NaCl.
  5. Wash with five resin-bed volumes of endotoxin-free, ultrapure water.
  6. Equilibrate with five resin-bed volumes of endotoxin-free buffer.
  7. Apply sample to the column at a flow rate of 10-15mL/hr and collect the flow-through (see the Important Product Information Section).
  8. Elute the protein by adding endotoxin-free buffer and collecting fractions; one or two resin-bed volume elutions are sufficient.
  9. Determine the endotoxin concentration of the processed sample. **Use caution to prevent sample contamination from dust or contaminated tubes subsequent to endotoxin removal.** Store solutions frozen or assay them before use to ensure sterility.
  10. Regenerate the resin as described in Steps 3-5 and store the column in 20% ethanol at 2-8°C.

## Endotoxin Removal Procedure using the Batch Method with Spin Columns

- Regenerate the resin before the first use and after each subsequent use. For regeneration and equilibration (steps 1-10), use regeneration buffer in 2mL volumes for the 0.25mL column, 3.5mL volumes for the 0.5mL column and 8mL volumes for the 1mL column.
  - Wear appropriate gloves while handling the spin columns. Use caution to prevent sample contamination during the endotoxin-removal procedure.
1. Equilibrate the spin column to room temperature.
  2. Twist off the column's bottom closure and loosen the top cap. Place the spin column into a collection tube. Centrifuge the column at  $500 \times g$  for 1 minute to remove the storage solution. Discard the storage solution.
  3. Remove the column cap and insert the bottom plug. To regenerate, add 0.2N NaOH, replace the cap, invert the column several times until the resin is suspended in the solution and incubate overnight at room temperature, **OR** add 0.2N NaOH in 95% ethanol, replace the cap, invert the column several times until the resin is suspended in the solution and incubate 1-2 hours at room temperature.
  4. Loosen the cap and remove the bottom plug. Place the column in a collection tube and centrifuge at  $500 \times g$  for 1 minute to remove the solution. Discard the solution.
  5. Remove the cap and insert the bottom plug. Add 2M NaCl, replace the cap and invert the column several times until the resin is suspended in the solution.
  6. Loosen the cap and remove the bottom plug. Place the column in a collection tube and centrifuge at  $500 \times g$  for 1 minute to remove the solution. Discard the solution.
  7. Remove the cap and insert the bottom plug. Add endotoxin-free, ultrapure water. Replace the cap and invert the column several times until the resin is suspended in the solution.
  8. Loosen the cap and remove the bottom plug. Place the column in a collection tube and centrifuge at  $500 \times g$  for 1 minute to remove the solution. Discard the solution.
  9. Remove the cap and insert the bottom plug. Add endotoxin-free buffer, replace the cap and invert the column several times until the resin is suspended in the solution.

10. Loosen the cap and remove the bottom plug. Place the column in a collection tube and centrifuge at  $500 \times g$  for 1 minute to remove the solution. Discard the solution. Repeat steps 9 and 10 two additional times.
11. Remove the cap and insert the bottom plug. Add the sample to the resin, replace the cap and invert the column several times until the resin is suspended in the solution.
12. Incubate the column with gentle end-over-end mixing at room temperature or  $4^{\circ}\text{C}$  for 1 hour. Incubation time can be extended depending on the sample type and requirements (see the Important Product Information Section).
13. Loosen the cap and remove the bottom plug. Place column in a collection tube and centrifuge at  $500 \times g$  for 1 minute to collect the sample.
14. Determine the endotoxin concentration of the processed sample. **Use caution to prevent sample contamination after endotoxin removal.** Store solutions at  $-20^{\circ}\text{C}$  or assay before use to ensure sterility.
15. Regenerate the resin as described in step 3 and store the column in 20% ethanol at  $2-8^{\circ}\text{C}$ .

## Troubleshooting

Problem	Possible Cause	Solution
Low endotoxin removal efficiency	Sample pH was not within a neutral range	Adjust sample to pH 6-8
	Incubation time was not sufficient	Increase the incubation time for the batch method
	Endotoxin was bound to the target protein	Recycle the sample through the column several times
	The removal or detection system was contaminated by extrinsic LPS	Use endotoxin-free labware and buffers
Low protein/sample recovery	Target protein aggregated with endotoxin and was removed	Increase NaCl concentration in the sample and equilibration buffer to 0.4M
	Nonspecific binding of sample to the resin	

## Related Thermo Scientific Products

<b>88282</b>	<b>Pierce LAL Chromogenic Endotoxin Quantitation Kit</b>
<b>89896, 7, 8</b>	<b>Pierce Centrifuge Columns, (2mL, 5mL and 10mL, respectively), 25/pkg</b>
<b>23225</b>	<b>Pierce BCA Protein Assay Kit</b>
<b>22660</b>	<b>Pierce 660nm Protein Assay</b>

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# SAFETY DATA SHEET

**Thermo**  
SCIENTIFIC

## Endotoxin Removal Resin

### Section 1. Identification

**GHS product identifier** : Endotoxin Removal Resin  
**Other means of identification** : Endotoxin Removal Resin, High Capacity; Endotoxin Removal Resin, Ultra Capacity; Pierce High Capacity Endotoxin Removal Resin  
**Product type** : Liquid.  
**Product code** : 0088270 0088271 0088272 0088273 0088274 0088275 0088276 0088277 1862581 1862582 1896231 1896254  
**SDS #** : 8743  
**Chemical formula** : Not applicable.  
**CAS #** : Not applicable.

#### Relevant identified uses of the substance or mixture and uses advised against

Not applicable.

**Supplier's details** : Thermo Fisher Scientific  
Pierce Biotechnology  
P.O. Box 117  
Rockford, IL 61105  
United States  
815.968.0747 or  
800.874.3723  
7 AM - 5 PM Central Time (GMT -06:00)

**Emergency telephone number (with hours of operation)** : CHEMTREC: 800.424.9300  
Outside US: 703.527.3887

### Section 2. Hazards identification

**OSHA/HCS status** : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

**Classification of the substance or mixture** : FLAMMABLE LIQUIDS - Category 4  
SKIN CORROSION/IRRITATION - Category 2  
SERIOUS EYE DAMAGE/ EYE IRRITATION - Category 2A  
CARCINOGENICITY - Category 2  
TOXIC TO REPRODUCTION (Fertility) - Category 1A  
TOXIC TO REPRODUCTION (Unborn child) - Category 1B

#### GHS label elements

**Hazard pictograms** :



**Signal word** : Danger

**Hazard statements** : Combustible liquid.  
Causes serious eye irritation.  
Causes skin irritation.  
May damage fertility or the unborn child.  
Suspected of causing cancer.

#### Precautionary statements

**Prevention** : Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Use personal protective equipment as required. Wear protective gloves. Wear eye or face protection. Keep away from flames and hot surfaces. - No smoking. Wash hands thoroughly after handling.

## Section 2. Hazards identification

**Response** : IF exposed or concerned: Get medical attention. IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing. Wash contaminated clothing before reuse. If skin irritation occurs: Get medical attention. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical attention.

**Storage** : Store locked up. Store in a well-ventilated place. Keep cool.

**Disposal** : Dispose of contents and container in accordance with all local, regional, national and international regulations.

**Hazards not otherwise classified** : None known.

## Section 3. Composition/information on ingredients

**Substance/mixture** : Mixture

**Other means of identification** : Endotoxin Removal Resin, High Capacity; Endotoxin Removal Resin, Ultra Capacity; Pierce High Capacity Endotoxin Removal Resin

### CAS number/other identifiers

**CAS number** : Not applicable.

<b>Ingredient name</b>	<b>%</b>	<b>CAS number</b>
ethanol	10 - 20	64-17-5

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

**There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.**

Occupational exposure limits, if available, are listed in Section 8.

## Section 4. First aid measures

### Description of necessary first aid measures

**Eye contact** : Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention.

**Inhalation** : Remove victim to fresh air and keep at rest in a position comfortable for breathing. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

**Skin contact** : Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Wash contaminated clothing thoroughly with water before removing it, or wear gloves. Continue to rinse for at least 10 minutes. Get medical attention. Wash clothing before reuse. Clean shoes thoroughly before reuse.

**Ingestion** : Wash out mouth with water. Remove dentures if any. Remove victim to fresh air and keep at rest in a position comfortable for breathing. If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Stop if the exposed person feels sick as vomiting may be dangerous. Do not induce vomiting unless directed to do so by medical personnel. If vomiting occurs, the head should be kept low so that vomit does not enter the lungs. Get medical attention. Never give anything by mouth to an unconscious person. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

### Most important symptoms/effects, acute and delayed

#### Potential acute health effects

**Eye contact** : Causes serious eye irritation.

**Inhalation** : No known significant effects or critical hazards.

## Section 4. First aid measures

- Skin contact** : Causes skin irritation.
- Ingestion** : Irritating to mouth, throat and stomach.
- Over-exposure signs/symptoms**
- Eye contact** : Adverse symptoms may include the following:  
pain or irritation  
watering  
redness
- Inhalation** : Adverse symptoms may include the following:  
reduced fetal weight  
increase in fetal deaths  
skeletal malformations
- Skin contact** : Adverse symptoms may include the following:  
irritation  
redness  
reduced fetal weight  
increase in fetal deaths  
skeletal malformations
- Ingestion** : Adverse symptoms may include the following:  
reduced fetal weight  
increase in fetal deaths  
skeletal malformations

### Indication of immediate medical attention and special treatment needed, if necessary

- Notes to physician** : Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.
- Specific treatments** : No specific treatment.
- Protection of first-aiders** : No action shall be taken involving any personal risk or without suitable training. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Wash contaminated clothing thoroughly with water before removing it, or wear gloves.

See toxicological information (Section 11)

## Section 5. Fire-fighting measures

### Extinguishing media

- Suitable extinguishing media** : Use dry chemical, CO<sub>2</sub>, water spray (fog) or foam.
- Unsuitable extinguishing media** : Do not use water jet.

- Specific hazards arising from the chemical** : Combustible liquid. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion. Runoff to sewer may create fire or explosion hazard.

- Hazardous thermal decomposition products** : Decomposition products may include the following materials:  
carbon dioxide  
carbon monoxide

- Special protective actions for fire-fighters** : Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool.

- Special protective equipment for fire-fighters** : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.



## Section 6. Accidental release measures

### Personal precautions, protective equipment and emergency procedures

- For non-emergency personnel** : No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Shut off all ignition sources. No flares, smoking or flames in hazard area. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.
- For emergency responders** : If specialised clothing is required to deal with the spillage, take note of any information in Section 8 on suitable and unsuitable materials. See also the information in "For non-emergency personnel".

- Environmental precautions** : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

### Methods and materials for containment and cleaning up

- Small spill** : Stop leak if without risk. Move containers from spill area. Use spark-proof tools and explosion-proof equipment. Dilute with water and mop up if water-soluble. Alternatively, or if water-insoluble, absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
- Large spill** : Stop leak if without risk. Move containers from spill area. Use spark-proof tools and explosion-proof equipment. Approach release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see Section 13). Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilled product. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

## Section 7. Handling and storage

### Precautions for safe handling

- Protective measures** : Put on appropriate personal protective equipment (see Section 8). Avoid exposure - obtain special instructions before use. Avoid exposure during pregnancy. Do not handle until all safety precautions have been read and understood. Do not get in eyes or on skin or clothing. Do not ingest. Avoid breathing vapor or mist. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Keep in the original container or an approved alternative made from a compatible material, kept tightly closed when not in use. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use only non-sparking tools. Empty containers retain product residue and can be hazardous. Do not reuse container.
- Advice on general occupational hygiene** : Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.

- Conditions for safe storage, including any incompatibilities** : Store in accordance with local regulations. Store in a segregated and approved area. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see Section 10) and food and drink. Store locked up. Eliminate all ignition sources. Separate from oxidizing materials. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

## Section 8. Exposure controls/personal protection

### Control parameters

#### Occupational exposure limits

Ingredient name	Exposure limits
ethanol	<p><b>ACGIH TLV (United States, 2000).</b> TWA: 1880 mg/m<sup>3</sup> 8 hours.</p> <p><b>OSHA (United States, 0/1989).</b> CEIL: 7600 ppm TWA: 1000 ppm TWA: 1900 mg/m<sup>3</sup></p> <p><b>MSHA (United States).</b> TWA: 1900 mg/m<sup>3</sup></p> <p><b>NIOSH (United States, 0/1994).</b> TWA: 1000 ppm TWA: 1900 mg/m<sup>3</sup></p> <p><b>ACGIH (United States, 0/1996).</b> TWA: 1880 mg/m<sup>3</sup></p> <p><b>ACGIH (United States).</b> TWA: 1000 ppm</p> <p><b>ACGIH TLV (United States, 6/2013).</b> STEL: 1000 ppm 15 minutes.</p> <p><b>NIOSH REL (United States, 4/2013).</b> TWA: 1900 mg/m<sup>3</sup> 10 hours. TWA: 1000 ppm 10 hours.</p> <p><b>OSHA PEL (United States, 2/2013).</b> TWA: 1900 mg/m<sup>3</sup> 8 hours. TWA: 1000 ppm 8 hours.</p> <p><b>OSHA PEL 1989 (United States, 3/1989).</b> TWA: 1900 mg/m<sup>3</sup> 8 hours. TWA: 1000 ppm 8 hours.</p>

#### Appropriate engineering controls

- : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

#### Environmental exposure controls

- : Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

### Individual protection measures

#### Hygiene measures

- : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

#### Eye/face protection

- : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: chemical splash goggles.

#### Skin protection

##### Hand protection

- : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.

## Section 8. Exposure controls/personal protection

- Body protection** : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
- Other skin protection** : Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
- Respiratory protection** : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

## Section 9. Physical and chemical properties

### Appearance

- Physical state** : Liquid. [Slurry]
- Color** : White-grayish
- Odor** : Not available.
- Odor threshold** : Not available.
- pH** : Not available.
- Melting point** : Not available.
- Boiling point** : Not available.
- Flash point** : Closed cup: 76.667°C (170°F)
- Burning time** : Not applicable.
- Burning rate** : Not applicable.
- Evaporation rate** : Not available.
- Flammability (solid, gas)** : Not available.
- Lower and upper explosive (flammable) limits** : Not available.
- Vapor pressure** : Not available.
- Vapor density** : Not available.
- Relative density** : Not available.
- Solubility** : Insoluble in the following materials: cold water.
- Solubility in water** : Not available.
- Partition coefficient: n-octanol/water** : Not available.
- Auto-ignition temperature** : Not available.
- Decomposition temperature** : Not available.
- SADT** : Not available.
- Viscosity** : Not available.

## Section 10. Stability and reactivity

- Reactivity** : No specific test data related to reactivity available for this product or its ingredients.
- Chemical stability** : The product is stable.
- Possibility of hazardous reactions** : Under normal conditions of storage and use, hazardous reactions will not occur.
- Conditions to avoid** : Avoid all possible sources of ignition (spark or flame). Do not pressurize, cut, weld, braze, solder, drill, grind or expose containers to heat or sources of ignition.
- Incompatible materials** : Reactive or incompatible with the following materials:  
oxidizing materials

## Section 10. Stability and reactivity

**Hazardous decomposition products** : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

## Section 11. Toxicological information

### Information on toxicological effects

#### Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
ethanol	LC50 Inhalation Vapor LD50 Oral	Rat Rat	124700 mg/m <sup>3</sup> 7 g/kg	4 hours -

**Conclusion/Summary** : To the best of our knowledge, the toxicological properties of this product have not been thoroughly investigated.

#### Irritation/Corrosion

Product/ingredient name	Result	Species	Score	Exposure	Observation
ethanol	Eyes - Mild irritant	Rabbit	-	24 hours 500 milligrams	-
	Eyes - Moderate irritant	Rabbit	-	0.066666667 minutes 100 milligrams	-
	Eyes - Moderate irritant	Rabbit	-	100 microliters	-
	Eyes - Severe irritant	Rabbit	-	500 milligrams	-
	Skin - Mild irritant	Rabbit	-	400 milligrams	-
	Skin - Moderate irritant	Rabbit	-	24 hours 20 milligrams	-

#### Sensitization

Not available.

#### Mutagenicity

Product/ingredient name	Test	Experiment	Result
ethanol	DNA Damage	Subject: Bacteria	Positive
	DNA Damage	Subject: Bacteria	Positive
	Mutation in Microorganisms	Subject: Bacteria	Positive
	Mutation in Microorganisms	Subject: Bacteria	Positive
	Gene Conversion and Mitotic Recombination	Subject: Bacteria	Positive
	Sex chromosome loss and nondisjunction.	Subject: Insect	Positive
	Cytogenetic Analysis	Subject: Mammalian-Animal	Positive
	Cytogenetic Analysis	Subject: Mammalian-Animal	Positive
	Cytogenetic Analysis	Subject: Mammalian-Animal	Positive
	Cytogenetic Analysis	Subject: Mammalian-Animal	Positive
	DNA Adduct	Subject: Mammalian-Animal	Positive
	DNA Adduct	Subject: Mammalian-Animal	Positive
	DNA Damage	Subject: Mammalian-Animal	Positive
	Micronucleus Test	Subject: Mammalian-Animal	Positive
	-	Subject: Mammalian-Animal	Positive
	Other Mutation Test	Subject: Mammalian-Animal	Positive
	Systems	Subject: Mammalian-Animal	Positive
	Other Mutation Test	Subject: Mammalian-Animal	Positive
	Systems	Subject: Mammalian-Animal	Positive
	Sister Chromatid Exchange	Subject: Mammalian-Animal	Positive
	Specific Locus Test	Subject: Mammalian-Animal	Positive
	Sperm Morphology	Subject: Mammalian-Animal	Positive
	Cytogenetic Analysis	Subject: Mammalian-Human	Positive
	Cytogenetic Analysis	Subject: Mammalian-Human	Positive
	Cell: Germ	Subject: Mammalian-Human	Positive
	Micronucleus Test	Subject: Mammalian-Human	Positive

## Section 11. Toxicological information

	Micronucleus Test DNA Inhibition Cytogenetic Analysis Cytogenetic Analysis Cytogenetic Analysis Sister Chromatid Exchange	Cell: Somatic Subject: Mammalian-Human Subject: Mammalian-Human Subject: Mammalian-Human Subject: Mammalian-Human Subject: Mammalian-Human	Positive Positive Positive Positive Positive Positive
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### Carcinogenicity

Product/ingredient name	Result	Species	Dose	Exposure
ethanol	Equivocal - Oral - TD	Mouse	400 g/kg	57 weeks
	Equivocal - Unreported - TDLo	Mouse	120 g/kg	Intermittent 18 weeks
	Equivocal - Oral - TDLo	Mouse	320 mg/kg	Intermittent 50 weeks

### Classification

Product/ingredient name	OSHA	IARC	NTP
ethanol	+	1	-

### Reproductive toxicity

Product/ingredient name	Maternal toxicity	Fertility	Development toxin	Species	Dose	Exposure
ethanol	-	-	-	Mouse	Intraperitoneal: 2.9 g/kg	8 days
	-	-	-	Mouse	Intraperitoneal: 2900 mg/kg	8 days
	-	-	-	Dog - Male	Unreported: 100 mg/kg	1 days
	-	Positive	-	Rat	Intraperitoneal: 600 mg/kg	15 days
	Positive	-	-	Mammal - species unspecified	Oral: 206 g/kg	-
	-	Positive	-	Rat - Male	Unreported: 400 mg/kg	1 days
	-	-	Positive	Mouse	Intraperitoneal: 15 g/kg	8 days
	-	Positive	-	Rat - Female	Unreported: 2400 mg/kg	10 days
	-	Positive	-	Woman - Female	Unreported: 200 mg/kg	5 days
	-	Positive	-	Dog	Oral: 221 g/kg	-
	-	Positive	-	Mammal - species unspecified	Oral: 78 g/kg	-
	-	-	Positive	Mouse	Intraperitoneal: 22.8 g/kg	8 days
	-	-	Positive	Mouse	Intraperitoneal: 5.8 g/kg	5 days
	-	-	Positive	Rat	Intraperitoneal: 600 mg/kg	15 days
	-	Positive	Positive	Mouse	Intraperitoneal: 2900 mg/kg	8 days
	-	-	Positive	Mammal - species unspecified	Intravenous	-
	-	-	-	Mouse - Male	Oral: 1680 g/kg	70 days

### Teratogenicity

## Section 11. Toxicological information

Product/ingredient name	Result	Species	Dose	Exposure
ethanol	Positive - Oral	Woman - Female	41 g/kg	-

### Specific target organ toxicity (single exposure)

Not available.

### Specific target organ toxicity (repeated exposure)

Not available.

### Aspiration hazard

Not available.

**Information on the likely routes of exposure** : Routes of entry anticipated: Oral, Dermal, Inhalation.

### Potential acute health effects

- Eye contact** : Causes serious eye irritation.
- Inhalation** : No known significant effects or critical hazards.
- Skin contact** : Causes skin irritation.
- Ingestion** : Irritating to mouth, throat and stomach.

### Symptoms related to the physical, chemical and toxicological characteristics

- Eye contact** : Adverse symptoms may include the following:  
pain or irritation  
watering  
redness
- Inhalation** : Adverse symptoms may include the following:  
reduced fetal weight  
increase in fetal deaths  
skeletal malformations
- Skin contact** : Adverse symptoms may include the following:  
irritation  
redness  
reduced fetal weight  
increase in fetal deaths  
skeletal malformations
- Ingestion** : Adverse symptoms may include the following:  
reduced fetal weight  
increase in fetal deaths  
skeletal malformations

### Delayed and immediate effects and also chronic effects from short and long term exposure

#### Short term exposure

- Potential immediate effects** : Not available.
- Potential delayed effects** : Not available.

#### Long term exposure

- Potential immediate effects** : Not available.
- Potential delayed effects** : Not available.

### Potential chronic health effects

Not available.

- General** : No known significant effects or critical hazards.
- Carcinogenicity** : Suspected of causing cancer. Risk of cancer depends on duration and level of exposure.
- Mutagenicity** : No known significant effects or critical hazards.
- Teratogenicity** : May damage the unborn child.

## Section 11. Toxicological information

**Developmental effects** : No known significant effects or critical hazards.  
**Fertility effects** : May damage fertility.

### Numerical measures of toxicity

#### Acute toxicity estimates

Not available.

## Section 12. Ecological information

### Toxicity

Product/ingredient name	Result	Species	Exposure
ethanol	Acute EC50 17.921 mg/l Marine water	Algae - Ulva pertusa	96 hours
	Acute EC50 2000 µg/l Fresh water	Daphnia - Daphnia magna	48 hours
	Acute LC50 25500 µg/l Marine water	Crustaceans - Artemia franciscana - Larvae	48 hours
	Acute LC50 42000 µg/l Fresh water	Fish - Oncorhynchus mykiss	4 days
	Chronic NOEC 4.995 mg/l Marine water	Algae - Ulva pertusa	96 hours
	Chronic NOEC 0.375 µl/L Fresh water	Fish - Gambusia holbrooki - Larvae	12 weeks

### Persistence and degradability

Product/ingredient name	Aquatic half-life	Photolysis	Biodegradability
ethanol	-	-	Readily

### Bioaccumulative potential

Product/ingredient name	LogP <sub>ow</sub>	BCF	Potential
ethanol	-0.35	0.66	low

### Mobility in soil

**Soil/water partition coefficient (K<sub>oc</sub>)** : Not available.

**Other adverse effects** : No known significant effects or critical hazards.

## Section 13. Disposal considerations

**Disposal methods** : The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Care should be taken when handling emptied containers that have not been cleaned or rinsed out. Empty containers or liners may retain some product residues. Vapor from product residues may create a highly flammable or explosive atmosphere inside the container. Do not cut, weld or grind used containers unless they have been cleaned thoroughly internally. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

## Section 14. Transport information

	DOT Classification	IATA
UN number	NA1993	Not regulated.
UN proper shipping name	Combustible liquids, n.o.s. (ethanol)	-
Transport hazard class(es)	Combustible liquid.	-
Packing group	III	-
Environmental hazards	No.	No.
Additional information	Non-bulk packages (less than or equal to 119 gal) of combustible liquids are not regulated as hazardous materials.	-

**Special precautions for user** : **Transport within user's premises:** always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.

**Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code** : Not available.

## Section 15. Regulatory information

**U.S. Federal regulations** : **TSCA 8(a) CDR Exempt/Partial exemption:** Not determined  
**United States inventory (TSCA 8b):** All components are listed or exempted.

**Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs)** : Not listed

**Clean Air Act Section 602 Class I Substances** : Not listed

**Clean Air Act Section 602 Class II Substances** : Not listed

**DEA List I Chemicals (Precursor Chemicals)** : Not listed

**DEA List II Chemicals (Essential Chemicals)** : Not listed

### SARA 302/304

#### Composition/information on ingredients

No products were found.

**SARA 304 RQ** : Not applicable.

### SARA 311/312

**Classification** : Fire hazard  
 Immediate (acute) health hazard  
 Delayed (chronic) health hazard

#### Composition/information on ingredients



## Section 15. Regulatory information

Name	%	Fire hazard	Sudden release of pressure	Reactive	Immediate (acute) health hazard	Delayed (chronic) health hazard
ethanol	10 - 20	Yes.	No.	No.	Yes.	Yes.

### State regulations

- Massachusetts** : The following components are listed: CELLULOSE; ETHYL ALCOHOL
- New York** : None of the components are listed.
- New Jersey** : The following components are listed: CELLULOSE; ETHYL ALCOHOL; ALCOHOL
- Pennsylvania** : The following components are listed: CELLULOSE; DENATURED ALCOHOL
- California Prop. 65**

**WARNING:** This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.

Ingredient name	Cancer	Reproductive	No significant risk level	Maximum acceptable dosage level
ethanol	No.	Yes.	No.	No.

**Canada inventory** : All components are listed or exempted.

### International regulations

- International lists** :
- Australia inventory (AICS):** All components are listed or exempted.
  - China inventory (IECSC):** All components are listed or exempted.
  - Japan inventory:** All components are listed or exempted.
  - Korea inventory:** All components are listed or exempted.
  - Malaysia Inventory (EHS Register):** Not determined.
  - New Zealand Inventory of Chemicals (NZIoC):** All components are listed or exempted.
  - Philippines inventory (PICCS):** All components are listed or exempted.
  - Taiwan inventory (CSNN):** Not determined.

**Chemical Weapons Convention List Schedule I Chemicals** : Not listed

**Chemical Weapons Convention List Schedule II Chemicals** : Not listed

**Chemical Weapons Convention List Schedule III Chemicals** : Not listed

## Section 16. Other information

### Hazardous Material Information System (U.S.A.)

Health 2  
 Chronic Health Hazard \*  
 Flammability 2  
 Physical hazards 0

### National Fire Protection Association (U.S.A.)

Health 2  
 Flammability 2  
 Instability/Reactivity 0  
 Special

The customer is responsible for determining the PPE code for this material.

## Section 16. Other information

**Caution:** HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings are not required on SDSs under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered mark of the National Paint & Coatings Association (NPCA). HMIS® materials may be purchased exclusively from J. J. Keller (800) 327-6868.

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Copyright ©2001, National Fire Protection Association, Quincy, MA 02269. This warning system is intended to be interpreted and applied only by properly trained individuals to identify fire, health and reactivity hazards of chemicals. The user is referred to certain limited number of chemicals with recommended classifications in NFPA 49 and NFPA 325, which would be used as a guideline only. Whether the chemicals are classified by NFPA or not, anyone using the 704 systems to classify chemicals does so at their own risk.

### History

<b>Date of printing</b>	: 2/17/2014.
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<b>Version</b>	: 1
<b>Prepared by</b>	: MSDS (Regulatory Specialist)
<b>Key to abbreviations</b>	: ATE = Acute Toxicity Estimate BCF = Bioconcentration Factor GHS = Globally Harmonized System of Classification and Labelling of Chemicals IATA = International Air Transport Association IBC = Intermediate Bulk Container IMDG = International Maritime Dangerous Goods LogPow = logarithm of the octanol/water partition coefficient MARPOL 73/78 = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution) UN = United Nations

**References** : Not available.

☑ Indicates information that has changed from previously issued version.

### Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.

Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

# POROS™ HIC Resins: Ethyl, Benzyl, and Benzyl Ultra

Pub. No. 100063752 Rev. A

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

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## Product information

### Product description

POROS™ Hydrophobic Interaction Chromatography (HIC) resins are rigid, 50-μL polymeric resins with a range of hydrophobic functionalities for the purification of antibody fragments, antibody drug conjugates (ADCs), recombinant proteins, viruses, and other biomolecules. The resin backbone consists of crosslinked poly(styrene-divinylbenzene) with a unique pore structure that provides rapid mass transport and enables enhanced productivity. The particle surface is coated with a novel polymer coating, which is then further derivatized with a range of hydrophobic ligands for flexible purification process design.

POROS™ HIC resins are suitable for bind/elute and flow-through applications at lower salt concentrations. These resins have superior resolution capability, high capacity, and differentiating selectivity for a range of biomolecules, and this performance is independent of flow rate.

### Storage

Store resins at 2–30°C. Do not freeze.

## Specifications

**Table 1** Ligands, hydrophobicity, and applications

Resin	Ligand	Relative hydrophobicity <sup>[1]</sup>	Application
Ethyl	Novel ethyl	Low	Bind/elute mode to bind moderately to strongly hydrophobic molecules.
Benzyl	Low-density benzyl/aromatic	Moderate	Bind/elute or flow-through mode depending on the hydrophobicity of the molecule.
Benzyl Ultra	High-density benzyl/aromatic	High	Flow-through mode in lower salt concentration to bind impurities such as aggregates.

<sup>[1]</sup> Hydrophobicity results are based on lysozyme gradient elution. Column size: 0.66 cmD × 20 cmL; 1.7-M ammonium sulfate, 50-mM sodium phosphate pH 7.0; Gradient elution: 1.7-M ammonium sulfate/50 mM sodium phosphate pH 7.0 to 50-mM sodium phosphate pH 7.0 over 10 column volumes; Flow rate: 100 cm/hr.

**Table 2** Characteristics and stability

Characteristic	Description
Support matrix	Crosslinked poly(styrene-divinylbenzene)
Shipping solution	18% ethanol
Average particle size	50 μm
Mechanical resistance	100 bar (1450 psi, 10 MPa)
pH range	1–14
Ionic strength range	0 to 5 M, all common salts
Buffer additives	All common agents, including 1 M sodium hydroxide, 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents
Salts	Ammonium sulfate, sodium sulfate, sodium chloride, sodium acetate, sodium citrate and other common salts <b>IMPORTANT!</b> POROS™ Benzyl and POROS™ Benzyl Ultra are designed for use with lower salt concentration than traditional HIC resins. With some molecules, high salt concentration can cause poor recovery due to a strong interaction between the target and the ligand.
Solvents	Water, 0–100% alcohol, acetonitrile, 1 to 2 M acids (for example, acetic, hydrochloric, phosphoric), other common organic solvents Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), acetone, or benzyl alcohol.
Shrinkage/swelling	<1% from 1–100% solvent
Operating temperature	2–30°C Do not freeze

POROS™ HIC resins can be operated at high linear flow rates with a pressure drop that allows use with conventional low-pressure chromatography columns and systems. POROS™ HIC resins have linear and predictable pressure flow responses as column diameter increases (Figure 1 and Figure 2).

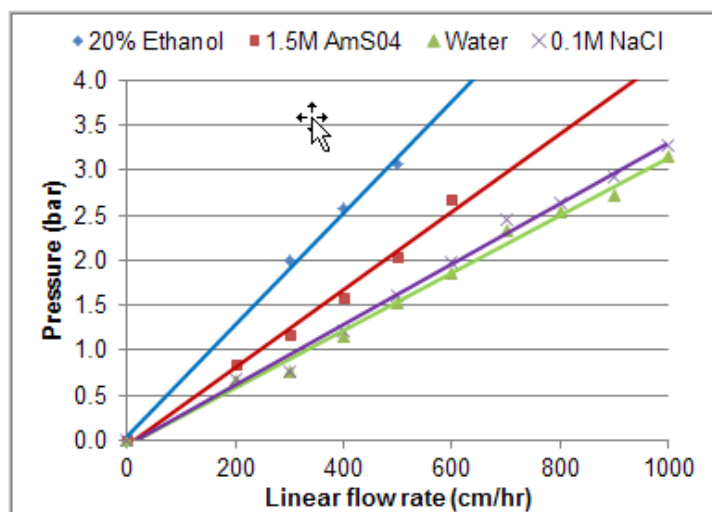


Fig. 1 Pressure-flow properties of POROS™ Ethyl resin—20-cm bed height

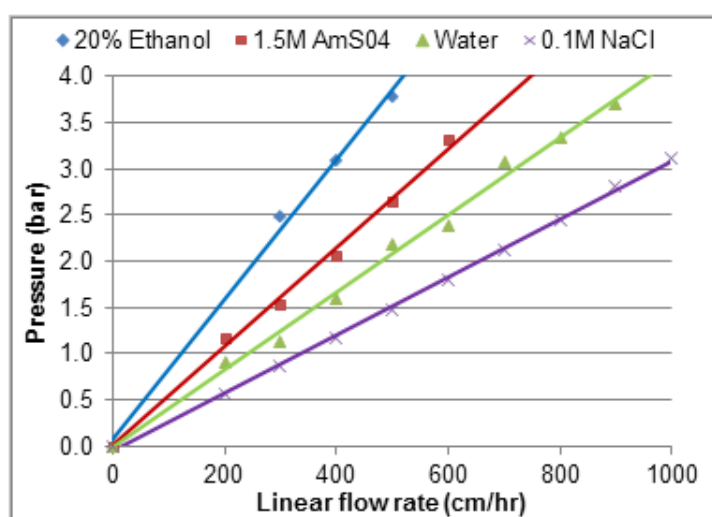


Fig. 2 Pressure-flow properties of POROS™ Benzyl Ultra resin—20-cm bed height

## Pack and qualify the column

### Packing guidelines

- Resins are supplied in 18% ethanol. For column packing, exchange the shipping solution with water to remove the ethanol.
- Resins are mechanically rigid and incompressible and can be packed effectively in low-pressure glass columns and in high-pressure stainless steel columns. The lack of wall support with increasing column diameter has minimal impact on chromatography performance because the beads support themselves, allowing for flexible column packing approaches and consistent and robust results. Columns can be packed with traditional flow pack, axial compression, or pack-in-place/stall pack packing methods.
- Standard 10–23  $\mu\text{m}$  screens (frits) can be used.

### Prepare slurry: lab-scale columns ( $\leq 100\text{ mL}$ )

Buffer-exchange using a 0.2–0.45  $\mu\text{m}$  bottle-top filter or sintered-glass filter:

- Transfer the required volume of resin slurry to the top of a bottle-top filter.
- Apply vacuum to remove the shipping solution.
- Resuspend the resin cake to the starting resin slurry volume with water. Mix with a plastic or rubber spatula. Do not grind the resin bed or tear the filter membrane.
- Repeat the vacuum and resuspension steps for a total of three exchanges.
- Resuspend the exchanged resin to the original slurry concentration, then proceed with column packing.
- Verify that the slurry concentration is 50–70% (see “Determine the slurry concentration” on page 2).
- If needed, adjust the slurry concentration to 50–70%.

### Prepare slurry: lab-scale and larger scale columns ( $> 100\text{ mL}$ )

Buffer-exchange using repeated gravity settling:

- Allow the resin to settle in the shipping container. Settling requires  $> 8$  hours because the density of the resin is approximately that of water.
- Carefully decant the supernatant. Do not disturb the bed. Some particles/turbidity may be present in the decant as beads slough off the settled bed or come loose from the carboy side walls. This is not problematic.
- Replace the supernatant with the same volume of the desired packing solution.
- Replace the supernatant with the same volume of water.
- Resuspend the resin by gentle agitation, then allow the resin to settle by gravity.
- Repeat steps 1 to 4 two to three times to thoroughly exchange into water.
- Verify that the slurry concentration is 50–70% (see “Determine the slurry concentration” on page 2).
- If needed, adjust the slurry concentration to 50–70%.

### Determine the slurry concentration

- Separate the slurry using either of the following methods.
  - Gravity settling**—Add 100 mL of slurry in water to a 100-mL graduated cylinder, then allow to settle for  $> 72$  hours.
 

**Note:** The time that POROS™ HIC resins take to gravity settle can be inconsistent due to hydrophobicity. We recommend using the centrifugation method for faster, more consistent results.
  - Centrifugation**—Add 10 mL of slurry in water to three (3) 15-mL conical tubes. Centrifuge at 3,000 rpm for 10 minutes at 20°C with the brake off. Remove the tubes from the centrifuge and allow the tubes to sit for 5 minutes before determining the concentration.
- Calculate the concentration: Volume of resin/total volume in the graduated cylinder or conical tube.

## Pack the column

When you adjust the flow rate to form the bed, you may observe some turbidity in the eluent as packing starts. Turbidity will clear as packing proceeds and 1–2 bed volumes of packing buffer pass through the column.

1. Determine the required slurry volume:

Example for a POROS™ Ethyl 40 cmD × 20 cmL 25-L column using slurry with a 50% slurry ratio:

$25 \text{ L} / 0.56 \times 1.06 = 47.3 \text{ L}$  slurry required

The 1.06 packing factor above accounts for the difference in bed volume between a centrifuged bed in water and a 3-bar pressure-packed bed. Use a 1.12 packing factor for POROS™ Benzyl and POROS™ Benzyl Ultra.

2. Ensure that the column outlet is closed and plumbed directly to waste. Do not connect the column outlet to the chromatography system. Plumbing into the system creates backpressure that fights against the inlet pressure trying to settle the bed and pack the column.
3. Ensure that the column is level and locked in place before starting the pack.
4. Deliver the required slurry volume to the column by hand or with a diaphragm pump, as dictated by your equipment and the intended packing procedure. Use a squirt bottle containing packing solution to remove any residual resin from the column wall.  
  
POROS™ resin beads have a skeletal density similar to the density of water and do not settle rapidly. Do not allow the resin to gravity-settle in the column before packing.
5. With the column inlet line connected to the system and the bottom outlet closed, bring the primed top flow adapter to 1–2 cm from the slurry level, then tighten the O-ring. Do not push up the resin and over the O-ring. Change the top valve to force the air and liquid out the top of the adapter and to waste using the bypass line. Continue to lower the adapter slowly to remove the bubbles from the top of the column. Do not allow large air bubbles between the top adaptor and the top of the resin slurry.
6. Change the valve back to flow through the system on the top, then open the column bottom.
7. Increase the flow rate to the maximum or desired flow rate and pressure obtainable with the equipment used.
8. After the bed is formed, bring the adapter into contact with the top of the bed without pushing the resin over the O-ring by closing the column outlet and displacing liquid through the top of the adapter to waste through the bypass line.

POROS™ resin does not shrink or swell, so an open headspace is not recommended.

9. Flow at the packing flow rate again for 1–2 CVs, taking note of the bed height at the desired pressure. Adjust the adapter again to the noted bed height by displacing the liquid through the top of the adapter and to waste.
10. After the column is packed, flow 2–3 CVs of packing solution through the packed bed at the operating flow rate to stabilize the bed.

The flow rate used should generate no more than 80% of the final packing pressure.

11. If you will reverse the flow of the column during operation, condition the column in upflow:
  - Flow 2–3 CVs in upflow at the operating flow rate.
  - Flow 2–3 CVs in downflow at the operating flow rate, then adjust the adapter if needed.
  - Flow 2 CVs after you adjust the adapter.

## Qualify the column

To qualify the integrity of a packed column, determine HETP (height equivalent to a theoretical plate) and asymmetry using a non-binding analyte (a “plug”).

### Recommended column qualification conditions

Condition	Recommendation
Flow rate	50 cm/hour
Equilibration buffer	Water
Plug solution	0.5 M sodium chloride
Plug volume	1% of column volume

### Guidelines for qualification

- Ensure uniform column plumbing:
  - Avoid using reducers to connect different tubing sizes.
  - Minimize and keep consistent the column tubing lengths between the plug solution to the column inlet and the column outlet to the detector(s).
- Equilibrate with at least 4 CVs of equilibration buffer before injection.

### Setting specifications

Qualification results depend on several factors, including the:

- Solutions and method used
- Scale
- Column hardware
- Chromatography system

After you define a column qualification procedure for a specific system (column plus chromatography system), base the qualification acceptance criteria on historical values and ranges instead of theoretical qualification results. Performing the column qualification method consistently and reproducibly is critical to obtaining meaningful results.

## Chromatography condition optimization

### General guidelines

Standardized conditions or platform-type evaluations are not recommended. Different HIC resins that are operated with the same process conditions can yield variable results.

When optimizing conditions:

- Test different loading and elution conditions to evaluate static binding capacity and yield based on the target molecule characteristics and process challenges.
- Limit static binding load incubation time to 15 minutes.
- Optimize the chromatography step for peak separation: Use conditions that remove some of the bound impurities during the flow-through/wash phase and that retain other bound impurities until elution during the strip and cleaning-in-place (CIP) steps.
- Use buffer salts and reagents of the highest purity.
- Filter (0.22 or 0.45 µm) all buffers, solutions, and load before use.

### Resin selection guidelines

- If the hydrophobicity of the target molecule is unknown:
  - Run a small-scale bind/elute gradient separation on POROS™ Benzyl resin to determine the elution conductivity of the target molecule, contaminants, and impurities.
  - Optimize conditions on POROS™ Benzyl Ultra resin (higher hydrophobicity) or POROS™ Ethyl resin (lower hydrophobicity).
  - If needed, continue to optimize conditions on POROS™ Benzyl resin (mid-range hydrophobicity).
- If the target molecule is hydrophobic, optimize conditions on POROS™ Ethyl resin or on POROS™ Benzyl with lower salt concentration.
- For flow-through applications where the target molecule is less hydrophobic, optimize conditions on POROS™ Benzyl or POROS™ Benzyl Ultra with lower salt concentration.

After you select the resin, continue to optimize other process conditions.

### Binding capacity and loading condition screening guidelines

Perform high-throughput static binding capacity testing in spin columns or in a 96-well plate to screen POROS™ HIC resin. Optimize loading conditions as needed.

**Note:** A 96-well high-throughput protocol is available on request. This protocol can be used to evaluate static binding capacity and to screen several resins and loading conditions per resin in a single 96-well plate.

### Bind/elute chromatography optimization guidelines

Binding conditions guidelines

- **Salt and salt concentration** — POROS™ HIC resins are designed to use less lyotropic salts and to bind at lower concentrations than traditional HIC resins with similar functional groups (Table 3).

**Table 3** Typical salts used in hydrophobic interaction chromatography in order of decreasing lyotropic ("salting out") effect and increasing chaotropic ("salting in") effect

Effect	Anionic salts	Cationic salts
Most lyotropic	C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> <sup>3-</sup>	NH <sub>4</sub> <sup>+</sup>
	PO <sub>4</sub> <sup>3-</sup>	Rb <sup>+</sup>
	SO <sub>4</sub> <sup>2-</sup>	K <sup>+</sup>
	CH <sub>3</sub> COO <sup>-</sup>	Na <sup>+</sup>
	Cl <sup>-</sup>	Cs <sup>+</sup>
	Br <sup>-</sup>	Li <sup>+</sup>
	NO <sub>3</sub> <sup>-</sup>	Mg <sup>2+</sup>
	ClO <sub>4</sub> <sup>-</sup>	Ca <sup>2+</sup>
Most chaotropic	I <sup>-</sup>	Ba <sup>2+</sup>

Note the following:

- You can perform initial binding experiments with lower salt concentrations than are typically used for HIC chromatography. For example, you can start with 25%, 50% and 75% of the salting out concentration, instead of the typical 10–15% lower than the salting out concentration.
- The optimized ionic salt concentration for the salts listed above can differ from ammonium sulfate, which has been traditionally used for HIC chromatography.
- Different salts can provide different selectivity.

Optimize the salt concentration for the target molecule by using a "salting out" experiment: increase the ionic strength of the loading buffer until the sample precipitates. Alternatively, you can measure increasing optical density of the loading buffer to detect aggregation (~350 nm, but this value may differ with each salt). Salts that are commonly used to perform salting out experiments are ammonium sulfate, sodium chloride, sodium citrate, sodium sulfate, and sodium acetate.

- **Buffer system**—Buffer systems are not as critical for HIC processes as they are for ion-exchange chromatography steps. Citrate, acetate, Bis-Tris propane, HEPES, MES, sodium phosphate, succinate, and Tris are commonly used.

The buffer system is typically dictated by the upstream purification step. When selecting the upstream buffer system, consider molecule stability in the buffer, binding optimization, and buffering capacity.

- **pH**—pH is not typically critical for HIC processes, but it can affect the binding strength and selectivity. Because pH effects are unpredictable on HIC, test a few pH values over the stability range of the target molecule.
- **Flow rate**—The target operating flow rate is flexible. Start optimization at 4-minute residence time (300 cm/hr in a 20-cmL column).
- **Temperature**—Temperature can significantly impact HIC performance. Perform all optimization at the final intended process temperature.

### Elution conditions guidelines

Start elution optimization with a gradient elution. Most often, after elution performance is determined, you can implement a step elution.

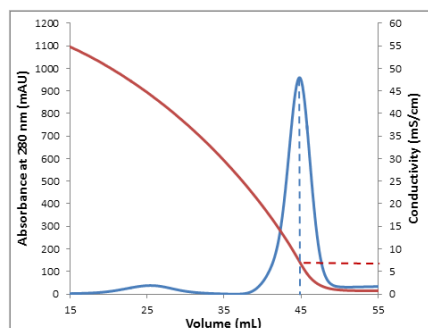
- **Salt gradient**—To determine where the target molecule and contaminants/ impurities elute, start with a 20-CV gradient from high salt to buffer only. To do so, assay fractions across the peaks (~1/10 CV). Based on this information, the process can be further optimized.
- **Dynamic binding capacity (DBC)**—Assess separation as a function of DBC. The maximum DBC depends on several factors, including sample solubility, column selectivity, buffer pH, and loading buffer conductivity.
- **Bed height**—Initial screening can be run with shorter bed heights and a constant residence time. Use the final desired bed height for scale up development (typically 15 cm to 30 cm).

### Flow-through chromatography optimization guidelines

An optional flow-through step can be used to remove trace product and process-related impurities such as aggregates from the target molecule. You can add the flow-through step as the second or third chromatography step for polishing in a downstream process. Different target molecules have different degrees of hydrophobicity and other biophysical characteristics. Therefore, it is essential to optimize the process conditions to achieve the desired aggregate clearance and recovery of the target molecule.

## Loading conditions guidelines

- **Initial study** — Perform an initial study using a decreasing salt gradient in bind/elute mode:
  - Load approximately 1 mg of protein per 1 mL of resin at 0.5–1.0 M sodium chloride (or other preferred salt), then elute using a gradient over 10 column volumes (CVs) in a buffered solution to determine aggregates, impurities, and target molecule elution profiles.
  - Use the elution conductivity at peak maximum to determine the highest approximate salt concentration that is required to remove impurities, but that allows the target molecule to flow through (Figure 3).



Start flow-through optimization at ~7 mS/cm

Fig. 3 Example screening chromatogram to obtain the ideal low salt condition for flow-through conditions. Process screening for a monoclonal antibody using POROS™ Benzyl Ultra resin in flow-through mode. Gradient: High conductivity to low conductivity using sodium citrate. Based on this chromatogram, the resin was further optimized in flow-through mode at low salt conditions starting at 7 mS/cm.

- **Buffer system** — Buffer systems are not as critical for HIC processes as they are for ion-exchange chromatography steps. Citrate, acetate, Bis-Tris propane, HEPES, MES, sodium phosphate, succinate, and Tris are commonly used.  
The buffer system is typically dictated by the upstream purification step. When selecting the upstream buffer system, consider molecule stability in the buffer, binding optimization, and buffering capacity.
- **pH** — pH is not typically critical for HIC processes, but it can affect the binding strength and selectivity. Because pH effects are unpredictable on HIC, test a few pH values over the stability range of the target molecule.
- **Flow rate** — The target operating flow rate is flexible. Good impurity binding has been demonstrated at flow rates up to 600 cm/hour on a 20 cmL column (1.6-minute residence time).
- **Temperature** — Temperature can significantly impact HIC performance. Perform all optimization at the final intended process temperature.
- **Dynamic binding capacity (DBC)** — A conservative starting point for DBC determination is 100–250 mg of the target molecule per mL of resin. Determine the DBC for each impurity by using breakthrough analysis under the desired load pH and conductivity conditions.
- **Bed height** — A bed height of 15 cm to 30 cm can be used for this step.

## Resin cleaning and storage

### Resin cleaning guidelines

- POROS™ resins can tolerate harsh cleaning conditions that allow acceptable column life.
- Clean the resin with 3 to 5 CVs of water followed by 3 to 5 CVs of 1 M NaOH.
- For more stringent cleaning, use 20% ethanol/1 M acetic acid.
- Other solutions may be required for column cleaning if the resin is used for capture chromatography.
- Degas more viscous solutions such as 1 M acetic acid or 20% ethanol before use on the column to avoid gassing out during operation.

**Note:** Low-level gassing out does not impact column performance.

### Store the resin

Store the resin in 20% ethanol or 0.1 M NaOH at 2–30°C.

## Ordering information

Table 4 POROS™ HIC bulk resins

Resin	Cat. No.	Amount	Product usage
Ethyl	A32552	10,000 mL	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
	A32553	5,000 mL	
	A32554	1,000 mL	
	A32555	250 mL	
	A32556	50 mL	
Benzyl	A32557	25 mL	For Research Use Only. Not for use in diagnostic procedures.
	A32558	10,000 mL	
	A32559	5,000 mL	
	A32560	1,000 mL	
	A32561	250 mL	
Benzyl Ultra	A32562	50 mL	For Research Use Only. Not for use in diagnostic procedures.
	A32563	25 mL	
	A32564	10,000 mL	
	A32565	5,000 mL	
	A32566	1,000 mL	
	A32567	250 mL	
	A32568	50 mL	
	A32569	25 mL	

## Support

For service and technical support, go to [thermofisher.com/poros](http://thermofisher.com/poros) or call toll-free in US: 1.800.831.6844.

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to [thermofisher.com/support](http://thermofisher.com/support), or contact your local Thermo Fisher Scientific representative.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

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**Manufacturer:** Life Technologies Corporation | 35 Wiggins Avenue | Bedford, MA 01730

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**Revision history:** Pub. No. 100063752

Revision	Date	Description
A	10 July 2017	New document.

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## Safety Data Sheet

### SECTION 1: Identification of the substance/mixture and of the company/undertaking

#### Identification of the substance or mixture

**Product code** A32561  
**Product name** POROS Benzyl Hydrophobic Interaction Chromatography Resin

#### Company/undertaking identification

Life Technologies Corporation  
5781 Van Allen Way  
PO Box 6482  
Carlsbad, CA 92008  
+1 760 603 7200

Life Technologies  
5250 Mainway Drive  
Burlington, ONT  
CANADA L7L 6A4  
800/263-6236

**24 hour Emergency Response for Hazardous Materials** Within the USA + Canada: 1-800-424-9300 and +1  
**[or Dangerous Goods] Incident. Spill, Leak, Fire,** 703-527-3887  
**Exposure, or Accident. Call CHEMTREC** Outside the USA + Canada: +1 703-741-5970

#### Country specific Emergency Number (if available):

CHEMTREC Brazil (Rio De Janeiro) +(55)-2139581449 (português)

**Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.**

### SECTION 2: Hazards identification

#### GHS - Classification

**Signal Word**  
WARNING

#### Hazard pictograms



**Health hazards**  
Not Hazardous

#### Physical hazards

GHS Physical Hazard 1	Flammable liquids
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**Revision date** 09-Oct-2017  
**Product code** A32561

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**Product name** POROS Benzyl Hydrophobic Interaction Chromatography Resin

**Environmental hazards**

Not Hazardous

**Hazard Statements**

H226 - Flammable liquid and vapor

**Precautionary Statements****Prevention**

P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking

P280 - Wear protective gloves/protective clothing/eye protection/face protection

P233 - Keep container tightly closed

P243 - Take action to prevent static discharges

P240 - Ground/bond container and receiving equipment

**Response**

P370 + P378 - In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction

P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower

**Storage**

P403 + P235 - Store in a well-ventilated place. Keep cool

**Disposal**

P501 - Dispose of contents/ container to an approved waste disposal plant

**Other hazards**

Not Applicable

**HMIS**

Health	0
Flammability	3
Reactivity	0

**SECTION 3: Composition/Information on ingredients**

Component	CAS-No	EINECS-No	Weight %
Ethyl alcohol 64-17-5 ( 15-22 )	64-17-5	200-578-6	15-22

We recommend handling all chemicals with caution.

## SECTION 4: First aid measures

### Description of first aid measures

<b>Skin contact</b>	Rinse with plenty of water . Immediate medical attention is not required.
<b>Eye contact</b>	Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do
<b>Ingestion</b>	Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.
<b>Inhalation</b>	Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.
<b>Notes to Physician</b>	Treat symptomatically.

### Most important symptoms and effects, both acute and delayed

H226 - Flammable liquid and vapor

### Indication of any immediate medical attention and special treatment needed

IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower.

## SECTION 5: Firefighting measures

### Extinguishing media

<b>Suitable extinguishing media</b>	Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
<b>Unsuitable extinguishing media</b>	No information available.

**Special hazards arising from the substance or mixture** Not known.

**Advice for fire-fighters** Standard procedure for chemical fires.

## SECTION 6: Accidental release measures

### Personal precautions, protective equipment and emergency procedures

Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. See Section 8 for more detail.

### Environmental precautions

No special environmental precautions required.

### Methods and material for containment and cleaning up

Soak up with inert absorbent material.

### Reference to other sections

See section 8 for more information.

## SECTION 7: Handling and storage

### Precautions for safe handling

Use personal protective equipment as required. No special handling advices are necessary.

### Conditions for safe storage, including any incompatibilities

Keep in a dry, cool and well-ventilated place. Keep in properly labeled containers.

### Specific end use(s)

Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.

## SECTION 8: Exposure controls/personal protection

### Control parameters

Chemical Name	OSHA PEL	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Ethyl alcohol	1000 ppm 1900 mg/m <sup>3</sup>	None	None	1000 ppm

**Engineering measures** Ensure adequate ventilation, especially in confined areas

### Exposure controls

#### Personal Protective Equipment

**Respiratory protection** In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

**Hand protection** Wear suitable gloves Glove material: Compatible chemical-resistant gloves.

**Eye protection** Tight sealing safety goggles

**Skin and Body Protection** Wear suitable protective clothing

**Hygiene measures** Handle in accordance with good industrial hygiene and safety practice

#### Environmental exposure controls

No special environmental precautions required.

## SECTION 9: Physical and chemical properties

### Information on basic physical and chemical properties

<b>Appearance</b>	suspension	
<b>Odor</b>	No data available	
<b>pH</b>	6-8	
<b>Melting point / melting range</b>	°C Mixture has not been tested	°F Mixture has not been tested
<b>Boiling point / boiling range</b>	°C Mixture has not been tested	°F Mixture has not been tested
<b>Flash point</b>	°C 36-49	°F 96.8-120.2
<b>Autoignition Temperature</b>	°C Mixture has not been tested	°F Mixture has not been tested
<b>Decomposition temperature</b>	°C Mixture has not been tested	°F Mixture has not been tested
<b>Evaporation rate</b>	No data available	
<b>Flammability (solid, gas)</b>	No data available	
<b>Upper explosion limit</b>	Mixture has not been tested	
<b>Lower explosion limit</b>	Mixture has not been tested	
<b>Vapor Pressure</b>	Mixture has not been tested	
<b>Relative density</b>	Mixture has not been tested	
<b>Specific gravity</b>	No data available	
<b>Solubility</b>	No data available	
<b>Partition coefficient: n-octanol/water</b>	No data available	
<b>Explosive properties</b>	Mixture has not been tested	
<b>Other information</b>	No data available	

## SECTION 10: Stability and reactivity

<b>Reactivity</b>	None known.
<b>Chemical stability</b>	Stable under normal conditions.
<b>Possibility of hazardous reactions</b>	Hazardous reaction has not been reported.
<b>Conditions to avoid</b>	No information available.
<b>Incompatible materials</b>	No dangerous reaction known under conditions of normal use.
<b>Hazardous decomposition products</b>	No data available.

## SECTION 11: Toxicological information

### Information on toxicological effects

Chemical Name	LD50 (oral, rat/mouse)	LD50 (dermal, rat/rabbit)	LC50 (inhalation, rat/mouse)
Ethyl alcohol	= 7060 mg/kg (Rat)	No data available	= 124.7 mg/L (Rat)

### Principal Routes of Exposure

<b>Irritation</b>	Conclusive but not sufficient for classification
<b>Corrosivity</b>	Conclusive but not sufficient for classification
<b>Sensitization</b>	Conclusive but not sufficient for classification
<b>STOT - Single Exposure</b>	Conclusive but not sufficient for classification
<b>STOT - Repeated Exposure</b>	Conclusive but not sufficient for classification
<b>Carcinogenicity</b>	Conclusive but not sufficient for classification
<b>Mutagenicity</b>	Conclusive but not sufficient for classification
<b>Reproductive toxicity</b>	May cause adverse reproductive effects - such as birth defect, miscarriages, or infertility
<b>Aspiration hazard</b>	Conclusive but not sufficient for classification

## SECTION 12: Ecological information

### Toxicity

The environmental impact of this product has not been fully investigated.

Chemical Name	Freshwater Algae Data	Water Flea Data	Freshwater Fish Species Data	Microtox Data	log Pow
Ethyl alcohol	No data available	Daphnia magna EC50=10800 mg/L (24 h) Daphnia magna EC50=2 mg/L (48 h) Daphnia magna LC509268 - 14221 mg/L (48 h)	No data available	No data available	logPow-0.32

**Persistence and degradability** No information available.

**Bioaccumulative potential** No information available.

### Results of PBT and vPvB assessment

No information available.

**Other adverse effects** No information available.



## SECTION 13: Disposal considerations

### Waste treatment methods

The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations

## SECTION 14: Transport information

### IATA / ADR / DOT-US / IMDG

This product is subjected to UN special provision A58 (144): An aqueous solution containing 24% or less alcohol by volume is not subject to IATA Dangerous Goods Regulations.

<b>UN number</b>	1170
<b>UN proper shipping name</b>	Ethanol Solution
<b>Transport hazard class(es)</b>	3
<b>Packing group</b>	III
<b>Environmental hazards</b>	Not Hazardous
<b>Special precautions for user</b>	Not Applicable

**Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code**  
Not Applicable.

## SECTION 15: Regulatory information

Component	US TSCA
Ethyl alcohol 64-17-5 ( 15-22 )	Listed

### US Federal Regulations

#### **SARA 313**

This product is not regulated by SARA.

#### **Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)**

This product does not contains HAPs.

### US State Regulations

#### **California Proposition 65**

This product contains the following Proposition 65 chemicals.

<u>Chemical Name</u>	<u>CAS-No</u>	<u>Weight %</u>	<u>Category</u>
Ethyl alcohol	64-17-5	15-22	Carcinogen Developmental

#### **WHMIS Hazard Class**

B3 - Combustible liquid



This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

## SECTION 16: Other information

<b>Reason for revision</b>	SDS sections updated.
<b>Revision number</b>	3
<b>Revision date</b>	09-Oct-2017

Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.

#### **References**

- ECHA: <http://echa.europa.eu/>
- TOXNET: <http://toxnet.nlm.nih.gov/>
- eChemPortal: <http://www.echemportal.org/>
- LOLI database: <https://www.chemadvisor.com/loli-database>

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE"

**Revision date** 09-Oct-2017  
**Product code** A32561

**Page** 8 / 9  
**Product name** POROS Benzyl Hydrophobic Interaction  
Chromatography Resin

**End of Safety Data Sheet**

---

**Revision date** 09-Oct-2017  
**Product code** A32561

**Page** 9 / 9  
**Product name** POROS Benzyl Hydrophobic Interaction  
Chromatography Resin

## SAFETY DATA SHEET

Version 5.2

Revision Date 01/11/2017

Print Date 04/10/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Adenine

Product Number : PHR1383

Brand : Sigma-Aldrich

CAS-No. : 73-24-5

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Acute toxicity, Oral (Category 3), H301

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word : Danger

Hazard statement(s)

H301 : Toxic if swallowed.

Precautionary statement(s)

P264 : Wash skin thoroughly after handling.

P270 : Do not eat, drink or smoke when using this product.

P301 + P310 + P330 : IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

P405 : Store locked up.

P501 : Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Molecular weight : 135.13 g/mol  
CAS-No. : 73-24-5  
EC-No. : 200-796-1

#### Hazardous ingredients according to Regulation (EC) No 1272/2008

Component		Classification	Concentration
<b>Adenine</b>			
CAS-No.	73-24-5	Acute Tox. 3; H301	<= 100 %
EC-No.	200-796-1		

For the full text of the H-Statements mentioned in this Section, see Section 16.

---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

#### In case of eye contact

Flush eyes with water as a precaution.

#### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust. For personal protection see section 8.

### 6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

## 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature 2 - 8 °C

Keep in a dry place. Keep in a dry place.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

Hazardous components without workplace control parameters

### 8.2 Exposure controls

#### Appropriate engineering controls

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

#### Personal protective equipment

##### Eye/face protection

Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Body Protection

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |                    |                                      |
|--------------------|--------------------------------------|
| a) Appearance      | Form: powder<br>Colour: light yellow |
| b) Odour           | No data available                    |
| c) Odour Threshold | No data available                    |
| d) pH              | No data available                    |

e) Melting point/freezing point	360 °C (680 °F)
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	No data available
n) Water solubility	No data available
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)

Other decomposition products - No data available

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 227 mg/kg

Remarks: Behavioral:Convulsions or effect on seizure threshold. Behavioral:Muscle weakness. Lungs, Thorax, or Respiration:Other changes.

Inhalation: No data available

Dermal: No data available

No data available

**Skin corrosion/irritation**

No data available

**Serious eye damage/eye irritation**

No data available

**Respiratory or skin sensitisation**

No data available

**Germ cell mutagenicity**

No data available

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

No data available

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available



---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

### DOT (US)

UN number: 2811      Class: 6.1      Packing group: III  
Proper shipping name: Toxic solids, organic, n.o.s. (Adenine)  
Reportable Quantity (RQ):  
Poison Inhalation Hazard: No

### IMDG

UN number: 2811      Class: 6.1      Packing group: III      EMS-No: F-A, S-A  
Proper shipping name: TOXIC SOLID, ORGANIC, N.O.S. (Adenine)

### IATA

UN number: 2811      Class: 6.1      Packing group: III  
Proper shipping name: Toxic solid, organic, n.o.s. (Adenine)

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Acute Health Hazard

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Adenine	73-24-5	

### New Jersey Right To Know Components

	CAS-No.	Revision Date
Adenine	73-24-5	

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

H301      Toxic if swallowed.

### HMIS Rating

Health hazard:	2
Chronic Health Hazard:	
Flammability:	0

Physical Hazard 0

**NFPA Rating**

Health hazard: 2

Fire Hazard: 0

Reactivity Hazard: 0

**Further information**

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

**Preparation Information**

Sigma-Aldrich Corporation

Product Safety – Americas Region

1-800-521-8956

Version: 5.2

Revision Date: 01/11/2017

Print Date: 04/10/2018

## SAFETY DATA SHEET

Version 5.2  
Revision Date 09/01/2017  
Print Date 03/24/2018

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : Ammonium sulfate, NF Grade

Product Number : RES1427A-A7

Brand : Sigma

CAS-No. : 7783-20-2

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture.

**2.2 GHS Label elements, including precautionary statements**

Not a hazardous substance or mixture.

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none**

---

**3. COMPOSITION/INFORMATION ON INGREDIENTS****3.1 Substances**

Formula : H<sub>8</sub>N<sub>2</sub>O<sub>4</sub>S

Molecular weight : 132.14 g/mol

CAS-No. : 7783-20-2

EC-No. : 231-984-1

No components need to be disclosed according to the applicable regulations.

---

**4. FIRST AID MEASURES****4.1 Description of first aid measures****General advice**

Consult a physician. Show this safety data sheet to the doctor in attendance.

**If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact**

Wash off with soap and plenty of water. Consult a physician.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Avoid breathing dust. For personal protection see section 8.

**6.2 Environmental precautions**

Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### Personal protective equipment

##### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |   |   |
|---|---|
| a) Appearance                                   | Form: crystalline<br>Colour: colourless |
| b) Odour  | odourless                               |
| c) Odour Threshold                              | No data available                       |
| d) pH   | 5.0 - 6 at 132 g/l at 25 °C (77 °F)     |
| e) Melting point/freezing point                 | > 280 °C (> 536 °F) - Decomposition     |
| f) Initial boiling point and boiling range      | No data available                       |
| g) Flash point                                  | No data available                       |
| h) Evaporation rate                             | No data available                       |
| i) Flammability (solid, gas)                    | The product is not flammable.           |
| j) Upper/lower flammability or explosive limits | No data available                       |
| k) Vapour pressure                              | No data available                       |
| l) Vapour density                               | No data available                       |
| m) Relative density                             | 1.770 g/cm <sup>3</sup>                 |
| n) Water solubility                             | 754 g/l at 25 °C (77 °F)                |

- |   |                   |
|---|-------------------|
| o) Partition coefficient: n-octanol/water | log Pow: -5.1     |
| p) Auto-ignition temperature              | No data available |
| q) Decomposition temperature              | No data available |
| r) Viscosity                              | No data available |
| s) Explosive properties                   | No data available |
| t) Oxidizing properties                   | No data available |

## 9.2 Other safety information

Bulk density	ca.1,200 kg/m3
--------------	----------------

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents, Strong bases

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Nitrogen oxides (NOx), Sulphur oxides  
Other decomposition products - No data available  
In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 4,250 mg/kg  
(OECD Test Guideline 401)

Inhalation: No data available

LD50 Dermal Dermal - Rat - > 2,000 mg/kg

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: No skin irritation

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: No eye irritation

#### Respiratory or skin sensitisation

Maximisation Test - Guinea pig

Result: Does not cause skin sensitisation.

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as

probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: BS4500000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish LC50 - Leuciscus idus (Golden orfe) - > 460 mg/l - 96 h

Toxicity to daphnia and other aquatic invertebrates LC50 - Daphnia (water flea) - 129 mg/l - 48 h

### 12.2 Persistence and degradability

The methods for determining biodegradability are not applicable to inorganic substances.

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION****SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

No SARA Hazards

**Massachusetts Right To Know Components**

	CAS-No.	Revision Date
Ammonium sulphate	7783-20-2	1993-04-24

**Pennsylvania Right To Know Components**

	CAS-No.	Revision Date
Ammonium sulphate	7783-20-2	1993-04-24

	CAS-No.	Revision Date
Ammonium sulphate	7783-20-2	1993-04-24

**New Jersey Right To Know Components**

	CAS-No.	Revision Date
Ammonium sulphate	7783-20-2	1993-04-24

**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

**16. OTHER INFORMATION****HMIS Rating**

Health hazard:	1
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

**NFPA Rating**

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

**Further information**

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.2

Revision Date: 09/01/2017

Print Date: 03/24/2018







## SAFETY DATA SHEET

according to the (US) Hazard Communication Standard (29 CFR 1910.1200)

Revision Date 09/16/2014

Version 3.0

### SECTION 1. Identification

#### Product identifier

Product number	801962
Product name	Brij® 35 for synthesis
CAS-No.	9002-92-0

#### Relevant identified uses of the substance or mixture and uses advised against

Identified uses	Chemical for synthesis
-----------------	------------------------

#### Details of the supplier of the safety data sheet

Company	EMD Millipore Corporation   290 Concord Road, Billerica, MA 01821, United States of America   General Inquiries: +1-978-715-4321   Monday to Friday, 9:00 AM to 4:00 PM Eastern Time (GMT-5)
---------	--

Emergency telephone	800-424-9300 CHEMTREC (USA) +1-703-527-3887 CHEMTREC (International) 24 Hours/day; 7 Days/week
---------------------	--

### SECTION 2. Hazards identification

#### GHS-Labeling

Not a dangerous substance according to GHS.

#### Other hazards

None known.

### SECTION 3. Composition/information on ingredients

Chemical nature	Alcohol
Formula	$(C_2H_4O)_n C_{12}H_{26}O$ $(C_2H_4O)_n C_{12}H_{26}O$ (Hill)

Remarks	No hazardous ingredients according to the OSHA Hazard Communication Standard 29 CFR 1910.1200.
---------	---

### SECTION 4. First aid measures

#### Description of first-aid measures

##### Inhalation

After inhalation: fresh air.

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according to the (US) Hazard Communication Standard (29 CFR 1910.1200)

Product number 801962  
Product name Brij® 35 for synthesis

Version 3.0

### *Skin contact*

After skin contact: wash off with plenty of water. Remove contaminated clothing.

### *Eye contact*

After eye contact: rinse out with plenty of water.

### *Ingestion*

After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

Never give anything by mouth to an unconscious person.

### **Most important symptoms and effects, both acute and delayed**

We have no description of any toxic symptoms.

### **Indication of any immediate medical attention and special treatment needed**

No information available.

---

## **SECTION 5. Fire-fighting measures**

### **Extinguishing media**

#### *Suitable extinguishing media*

Water, Carbon dioxide (CO<sub>2</sub>), Foam, Dry powder

#### *Unsuitable extinguishing media*

For this substance/mixture no limitations of extinguishing agents are given.

### **Special hazards arising from the substance or mixture**

Combustible.

Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapors possible in the event of fire.

### **Advice for firefighters**

#### *Special protective equipment for fire-fighters*

In the event of fire, wear self-contained breathing apparatus.

#### *Further information*

Prevent fire extinguishing water from contaminating surface water or the ground water system.

---

## **SECTION 6. Accidental release measures**

### **Personal precautions, protective equipment and emergency procedures**

Advice for non-emergency personnel: Avoid inhalation of dusts. Evacuate the danger area, observe emergency procedures, consult an expert.

Advice for emergency responders: Protective equipment see section 8.

### **Environmental precautions**

Do not empty into drains.

### **Methods and materials for containment and cleaning up**

Cover drains. Collect, bind, and pump off spills.

Observe possible material restrictions (see sections 7 and 10).

Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

SAFETY DATA SHEET  
according to the (US) Hazard Communication Standard (29 CFR 1910.1200)

Product number 801962  
Product name Brij® 35 for synthesis

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---

**SECTION 7. Handling and storage**

**Precautions for safe handling**

Observe label precautions.

**Conditions for safe storage, including any incompatibilities**

Tightly closed. Dry.

Store at +15°C to +25°C (+59°F to +77°F).

---

**SECTION 8. Exposure controls/personal protection**

**Exposure limit(s)**

Contains no substances with occupational exposure limit values.

**Engineering measures**

Technical measures and appropriate working operations should be given priority over the use of personal protective equipment.

**Individual protection measures**

Protective clothing should be selected specifically for the workplace, depending on concentration and quantity of the hazardous substances handled. The chemical resistance of the protective equipment should be inquired at the respective supplier.

*Hygiene measures*

Change contaminated clothing. Wash hands after working with substance.

*Eye/face protection*

Safety glasses

*Hand protection*

Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

*Respiratory protection*

required when dusts are generated.

Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

---

**SECTION 9. Physical and chemical properties**

Physical state	solid
Color	white
Odor	fatty odor
Odor Threshold	No information available.
pH	5.5 - 7.0 at 100 g/l 68 °F ( 20 °C)

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Product number	801962	Version 3.0
Product name	Brij® 35 for synthesis	

pour point	33 °C
Boiling point/boiling range	ca. 212 °F ( 100 °C) at 1,013 hPa
Flash point	> 300 °F ( > 149 °C)
Evaporation rate	No information available.
Flammability (solid, gas)	No information available.
Lower explosion limit	No information available.
Upper explosion limit	No information available.
Vapor pressure	< 1 hPa at 68 °F ( 20 °C)
Relative vapor density	No information available.
Density	1.05 g/cm³ at 68 °F ( 20 °C)
Relative density	No information available.
Water solubility	at 77 °F ( 25 °C) soluble
Partition coefficient: n-octanol/water	No information available.
Autoignition temperature	No information available.
Decomposition temperature	No information available.
Viscosity, dynamic	No information available.
Explosive properties	Not classified as explosive.
Oxidizing properties	none

---

**SECTION 10. Stability and reactivity**

**Reactivity**

The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

Forms explosive mixtures with air on intense heating.

**Chemical stability**

Sensitivity to light

## SAFETY DATA SHEET

according to the (US) Hazard Communication Standard (29 CFR 1910.1200)

Product number 801962  
Product name Brij® 35 for synthesis

Version 3.0

### Possibility of hazardous reactions

Violent reactions possible with:

Strong acids, Bases, Oxidizing agents

### Conditions to avoid

Strong heating.

A range from approx. 15 Kelvin below the flash point is to be rated as critical.

### Incompatible materials

no information available

### Hazardous decomposition products

no information available

---

## SECTION 11. Toxicological information

### Information on toxicological effects

#### *Likely route of exposure*

Eye contact, Skin contact, Ingestion

#### *Acute oral toxicity*

LD50 Rat: > 78,000 mg/kg (External MSDS)

#### *Skin irritation*

Rabbit

Result: No irritation

(External MSDS)

#### *Eye irritation*

Rabbit

Result: slight irritation

(External MSDS)

#### *Sensitization*

Patch test: human

Result: negative

(External MSDS)

#### *Specific target organ systemic toxicity - single exposure*

The substance or mixture is not classified as specific target organ toxicant, single exposure.

#### *Specific target organ systemic toxicity - repeated exposure*

The substance or mixture is not classified as specific target organ toxicant, repeated exposure.

#### *Aspiration hazard*

Regarding the available data the classification criteria are not fulfilled.

### Carcinogenicity

IARC

No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

OSHA

No ingredient of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential

SAFETY DATA SHEET  
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Product number	801962	Version 3.0
Product name	Brij® 35 for synthesis	

---

	carcinogen by OSHA.
NTP	No ingredient of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
ACGIH	No ingredient of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

**Further information**

Hazardous properties cannot be excluded but are unlikely when the product is handled appropriately.

Handle in accordance with good industrial hygiene and safety practice.

---

**SECTION 12. Ecological information**

**Ecotoxicity**

No information available.

**Persistence and degradability**

*Biodegradability*

89 %

OECD Test Guideline 301B

Readily biodegradable.

**Bioaccumulative potential**

No information available.

**Mobility in soil**

No information available.

---

**SECTION 13. Disposal considerations**

The information presented only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations. Disposal should be in accordance with applicable regional, national and local laws and regulations.

---

**SECTION 14. Transport information**

**Land transport (DOT)**

Not classified as dangerous in the meaning of transport regulations.

**Air transport (IATA)**

Not classified as dangerous in the meaning of transport regulations.

**Sea transport (IMDG)**

Not classified as dangerous in the meaning of transport regulations.

SAFETY DATA SHEET  
according to the (US) Hazard Communication Standard (29 CFR 1910.1200)

Product number 801962  
Product name Brij® 35 for synthesis

Version 3.0

---

**SECTION 15. Regulatory information**

**United States of America**

**SARA 313**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 302**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**Clean Water Act**

This product does not contain any Hazardous Substances listed under the U.S. CleanWater Act, Section 311, Table 116.4A.

This product does not contain any Hazardous Chemicals listed under the U.S. CleanWater Act, Section 311, Table 117.3.

**DEA List I**

Not listed

**DEA List II**

Not listed

**US State Regulations**

**Massachusetts Right To Know**

Remarks

No components are subject to the Massachusetts Right to Know Act.

**Pennsylvania Right To Know**

*Ingredients*

polymer of ethylene glycol and 1-dodecyl alcohol with >20 mol ethylene oxide

**New Jersey Right To Know**

*Ingredients*

polymer of ethylene glycol and 1-dodecyl alcohol with >20 mol ethylene oxide

**California Prop 65 Components**

This product does not contain any chemicals known to the State of California to cause cancer, birth, or any other reproductive defects.

**Notification status**

TSCA: All components of the product are listed in the TSCA-inventory.

DSL: All components of this product are on the Canadian DSL.

---

**SECTION 16. Other information**

**Training advice**

Provide adequate information, instruction and training for operators.

**Key or legend to abbreviations and acronyms used in the safety data sheet**



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---

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Used abbreviations and acronyms can be looked up at [www.wikipedia.org](http://www.wikipedia.org).

Revision Date 09/16/2014

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The information contained herein is based on the present state of our knowledge. It characterizes the product with regard to appropriate safety precautions. It does not represent a warranty of any product properties and we assume no liability for any loss or injury which may result from the use of this information. Users should conduct their own investigations to determine the suitability of the information.

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## SAFETY DATA SHEET

Version 5.0

Revision Date 01/17/2018

Print Date 04/10/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Chloramphenicol, United States Pharmacopeia  
(USP) Reference Standard

Product Number : 1107004

Brand : USP

CAS-No. : 56-75-7

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Carcinogenicity (Category 2), H351

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Warning

Hazard statement(s)

H351

Suspected of causing cancer.

Precautionary statement(s)

P201

Obtain special instructions before use.

P202

Do not handle until all safety precautions have been read and understood.

P280

Wear protective gloves/ protective clothing/ eye protection/ face protection.

P308 + P313

IF exposed or concerned: Get medical advice/ attention.

P405

Store locked up.

P501

Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

### 3. COMPOSITION/INFORMATION ON INGREDIENTS

#### 3.1 Substances

Molecular weight : 323.13 g/mol  
CAS-No. : 56-75-7

#### Hazardous components

Component	Classification	Concentration
Chloramphenicol	Carc. 2; H351	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

---

### 4. FIRST AID MEASURES

#### 4.1 Description of first aid measures

##### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

##### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

##### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

##### In case of eye contact

Flush eyes with water as a precaution.

##### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

#### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

#### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

### 5. FIREFIGHTING MEASURES

#### 5.1 Extinguishing media

##### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

#### 5.2 Special hazards arising from the substance or mixture

No data available

#### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

#### 5.4 Further information

No data available

---

### 6. ACCIDENTAL RELEASE MEASURES

#### 6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

For personal protection see section 8.

#### 6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Storage class (TRGS 510): 13: Non Combustible Solids

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Chloramphenicol	56-75-7	TWA	0.500000 mg/m3	USA. Workplace Environmental Exposure Levels (WEEL)

### 8.2 Exposure controls

#### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### Personal protective equipment

##### Eye/face protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Body Protection

Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |               |   |
|---------------|---|
| a) Appearance | Form: crystalline<br>Colour: light yellow |
|---------------|---|

b) Odour	No data available
c) Odour Threshold	No data available
d) pH	No data available
e) Melting point/freezing point	149 °C (300 °F)
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	No data available
n) Water solubility	practically insoluble
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

## 9.2 Other safety information

Solubility in other solvents	Ethanol
------------------------------	---------

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

acids, Acid chlorides, Acid anhydrides, Oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NO<sub>x</sub>), Hydrogen chloride gas

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 2,500 mg/kg

Inhalation: No data available

Dermal: No data available

LD50 Intraperitoneal - Rat - 1,811 mg/kg

LD50 Intraperitoneal - Mouse - 1,100 mg/kg

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

No data available

#### Respiratory or skin sensitisation

Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

#### Germ cell mutagenicity

Laboratory experiments have shown mutagenic effects.

Rat

Liver

DNA damage

Mouse

Cytogenetic analysis

#### Carcinogenicity

This product is or contains a component that has been reported to be probably carcinogenic based on its IARC, OSHA, ACGIH, NTP, or EPA classification.

Suspected human carcinogens

IARC: 2A - Group 2A: Probably carcinogenic to humans (Chloramphenicol)

NTP: RAHC - Reasonably anticipated to be a human carcinogen (Chloramphenicol)

RAHC - Reasonably anticipated to be a human carcinogen (Chloramphenicol)

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

#### Reproductive toxicity

No data available

No data available

#### Specific target organ toxicity - single exposure

No data available

#### Specific target organ toxicity - repeated exposure

No data available

#### Aspiration hazard

No data available

#### Additional Information

RTECS: Not available

Nausea, Headache, Vomiting

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Liver - Irregularities - Based on Human Evidence

Liver - Irregularities - Based on Human Evidence

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to daphnia and other aquatic invertebrates      EC50 - Daphnia magna (Water flea) - 345 mg/l - 48 h

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

#### DOT (US)

Not dangerous goods

#### IMDG

Not dangerous goods

#### IATA

Not dangerous goods

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Chronic Health Hazard

### Massachusetts Right To Know Components

Chloramphenicol

CAS-No.  
56-75-7

Revision Date  
1989-12-01

## Pennsylvania Right To Know Components

Chloramphenicol

CAS-No.  
56-75-7

Revision Date  
1989-12-01

Chloramphenicol

CAS-No.  
56-75-7

Revision Date  
1989-12-01

## New Jersey Right To Know Components

Chloramphenicol

CAS-No.  
56-75-7

Revision Date  
1989-12-01

## California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Carc.	Carcinogenicity
H351	Suspected of causing cancer.

### HMIS Rating

Health hazard:	1
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.0

Revision Date: 01/17/2018

Print Date: 04/10/2018



## SAFETY DATA SHEET

Version 5.2  
Revision Date 05/12/2015  
Print Date 04/08/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Citric acid, Anhydrous

Product Number : PHR1071  
Brand : Sigma-Aldrich

CAS-No. : 77-92-9

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Eye irritation (Category 2A), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word : Warning

Hazard statement(s)  
H319 : Causes serious eye irritation.

Precautionary statement(s)  
P264 : Wash skin thoroughly after handling.  
P280 : Wear eye protection/ face protection.  
P305 + P351 + P338 : IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.  
P337 + P313 : If eye irritation persists: Get medical advice/ attention.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Formula : C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>

Molecular weight : 192.12 g/mol  
CAS-No. : 77-92-9  
EC-No. : 201-069-1

#### Hazardous components

Component	Classification	Concentration
<b>Citric acid</b>		
	Eye Irrit. 2A; H319	<= 100 %

No components need to be disclosed according to the applicable regulations.  
For the full text of the H-Statements mentioned in this Section, see Section 16.

---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Move out of dangerous area. Consult a physician. Show this safety data sheet to the doctor in attendance.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

#### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

#### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

Carbon oxides

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

For personal protection see section 8.

### 6.2 Environmental precautions

Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs. Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Store at Room Temperature.

Storage class (TRGS 510): Non Combustible Solids

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### Personal protective equipment

##### Eye/face protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatrill® (KCL 740 / Aldrich Z677272, Size M)

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatrill® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

##### Body Protection

impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

**Respiratory protection**

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**Control of environmental exposure**

Do not let product enter drains.

---

**9. PHYSICAL AND CHEMICAL PROPERTIES****9.1 Information on basic physical and chemical properties**

a) Appearance	Form: crystalline Colour: white
b) Odour	No data available
c) Odour Threshold	No data available
d) pH	1.8 at ca.50 g/l at 25 °C (77 °F)
e) Melting point/freezing point	Melting point/freezing point: 155 - 157 °C (311 - 315 °F)
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	Lower explosion limit: 8 %(V)
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	No data available
n) Water solubility	383 g/l at 25 °C (77 °F)
o) Partition coefficient: n-octanol/water	log Pow: -1.639 at 20 °C (68 °F)
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

**9.2 Other safety information**

No data available

---

**10. STABILITY AND REACTIVITY****10.1 Reactivity**

No data available

**10.2 Chemical stability**

Stable under recommended storage conditions.

**10.3 Possibility of hazardous reactions**

No data available

#### 10.4 Conditions to avoid

No data available

#### 10.5 Incompatible materials

Oxidizing agents, Bases, Reducing agents, Nitrates

#### 10.6 Hazardous decomposition products

Other decomposition products - No data available

In the event of fire: see section 5

---

### 11. TOXICOLOGICAL INFORMATION

#### 11.1 Information on toxicological effects

##### Acute toxicity

LD50 Oral - Rat - 5,400 mg/kg  
(OECD Test Guideline 401)

Inhalation: No data available

LD50 Dermal - Rat - > 2,000 mg/kg  
(OECD Test Guideline 402)

No data available

##### Skin corrosion/irritation

Skin - Rabbit

Result: Mild skin irritation  
(OECD Test Guideline 404)

##### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Irritating to eyes.  
(OECD Test Guideline 405)

##### Respiratory or skin sensitisation

Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

##### Germ cell mutagenicity

No data available

##### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

##### Reproductive toxicity

No data available

No data available

##### Specific target organ toxicity - single exposure

No data available

##### Specific target organ toxicity - repeated exposure

No data available

##### Aspiration hazard

No data available

##### Additional Information

RTECS: GE7350000

Vomiting, Diarrhoea, Damage to tooth enamel., Dermatitis, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish                      mortality LC50 - *Leuciscus idus melanotus* - 440 mg/l - 48 h  
(OECD Test Guideline 203)

Toxicity to daphnia and      static test - *Daphnia magna* (Water flea) - 1,535 mg/l - 24 h  
other aquatic  
invertebrates

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

#### DOT (US)

Not dangerous goods

#### IMDG

Not dangerous goods

#### IATA

Not dangerous goods

---

## 15. REGULATORY INFORMATION

#### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

#### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

#### SARA 311/312 Hazards

Acute Health Hazard

#### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

#### Pennsylvania Right To Know Components

CAS-No.

Revision Date

Citric acid

77-92-9

## New Jersey Right To Know Components

Citric acid

CAS-No.  
77-92-9

Revision Date

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Eye Irrit.	Eye irritation
H319	Causes serious eye irritation.

### HMIS Rating

Health hazard:	2
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	2
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.2

Revision Date: 05/12/2015

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 4.5  
Revision Date 12/21/2015  
Print Date 04/08/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : D-(+)-Glucose monohydrate

Product Number : 49159  
Brand : Sigma-Aldrich

CAS-No. : 14431-43-7

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

## 2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Synonyms : Dextrosemonohydrate  
Glucose

Formula :  $C_6H_{12}O_6 \cdot H_2O$   
Molecular weight : 198.17 g/mol  
CAS-No. : 14431-43-7  
EC-No. : 218-914-5

No components need to be disclosed according to the applicable regulations.

---

4. FIRST AID MEASURES

## 4.1 Description of first aid measures

**If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.



**In case of skin contact**

Wash off with soap and plenty of water.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

Carbon oxides

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

**6.2 Environmental precautions**

Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

Keep in a dry place. Keep in a dry place.

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

**8. EXPOSURE CONTROLS/PERSONAL PROTECTION****8.1 Control parameters****Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

**8.2 Exposure controls****Appropriate engineering controls**

General industrial hygiene practice.

## Personal protective equipment

### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

#### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

#### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

### Control of environmental exposure

Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |  |                               |
|--|-------------------------------|
| a) Appearance                              | Form: powder<br>Colour: white |
| b) Odour                                   | No data available             |
| c) Odour Threshold                         | No data available             |
| d) pH                                      | No data available             |
| e) Melting point/freezing point            | No data available             |
| f) Initial boiling point and boiling range | No data available             |
| g) Flash point                             | No data available             |
| h) Evaporation rate                        | No data available             |
| i) Flammability (solid, gas)               | No data available             |
| j) Upper/lower flammability or             | No data available             |

explosive limits

- |   |                   |
|---|-------------------|
| k) Vapour pressure                        | No data available |
| l) Vapour density                         | No data available |
| m) Relative density                       | No data available |
| n) Water solubility                       | No data available |
| o) Partition coefficient: n-octanol/water | No data available |
| p) Auto-ignition temperature              | No data available |
| q) Decomposition temperature              | No data available |
| r) Viscosity                              | No data available |
| s) Explosive properties                   | No data available |
| t) Oxidizing properties                   | No data available |

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Other decomposition products - No data available  
In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

No data available

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

No data available

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

### **Carcinogenicity**

- IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
- ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
- NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
- OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

### **Reproductive toxicity**

No data available

No data available

### **Specific target organ toxicity - single exposure**

No data available

### **Specific target organ toxicity - repeated exposure**

No data available

### **Aspiration hazard**

No data available

### **Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

## **12. ECOLOGICAL INFORMATION**

### **12.1 Toxicity**

No data available

### **12.2 Persistence and degradability**

No data available

### **12.3 Bioaccumulative potential**

No data available

### **12.4 Mobility in soil**

No data available

### **12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### **12.6 Other adverse effects**

No data available

---

## **13. DISPOSAL CONSIDERATIONS**

### **13.1 Waste treatment methods**

#### **Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

#### **Contaminated packaging**

Dispose of as unused product.

---

## **14. TRANSPORT INFORMATION**

### **DOT (US)**

Not dangerous goods

### **IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION**

**SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

No SARA Hazards

**Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

**Pennsylvania Right To Know Components**

	CAS-No.	Revision Date
D-Glucose monohydrate	14431-43-7	

**New Jersey Right To Know Components**

	CAS-No.	Revision Date
D-Glucose monohydrate	14431-43-7	

**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

**16. OTHER INFORMATION**

**HMIS Rating**

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

**NFPA Rating**

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

**Further information**

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 4.5

Revision Date: 12/21/2015

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 5.5  
Revision Date 10/03/2017  
Print Date 04/07/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : *N,N*-Dimethylacrylamide

Product Number : 274135

Brand : Aldrich

CAS-No. : 2680-03-7

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Flammable liquids (Category 4), H227

Acute toxicity, Oral (Category 3), H301

Acute toxicity, Dermal (Category 3), H311

Serious eye damage (Category 1), H318

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Danger

Hazard statement(s)

H227

H301 + H311

H318

Combustible liquid.

Toxic if swallowed or in contact with skin

Causes serious eye damage.

Precautionary statement(s)

P210

P264

P270

P280

P301 + P310 + P330

P302 + P352 + P312

Keep away from heat/sparks/open flames/hot surfaces. No smoking.

Wash skin thoroughly after handling.

Do not eat, drink or smoke when using this product.

Wear protective gloves/ eye protection/ face protection.

IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

IF ON SKIN: Wash with plenty of soap and water. Call a POISON

P305 + P351 + P338 + P310	CENTER or doctor/ physician if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
P361	Remove/Take off immediately all contaminated clothing.
P363	Wash contaminated clothing before reuse.
P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction.
P403 + P235	Store in a well-ventilated place. Keep cool.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

## 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.1 Substances

Formula	: C <sub>5</sub> H <sub>9</sub> NO
Molecular weight	: 99.13 g/mol
CAS-No.	: 2680-03-7
EC-No.	: 220-237-5

#### Hazardous components

Component	Classification	Concentration
<b>N,N-Dimethylacrylamide</b>		
	Flam. Liq. 4; Acute Tox. 3; Eye Dam. 1; H227, H301 + H311, H318	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

#### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

#### If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

## **5.2 Special hazards arising from the substance or mixture**

No data available

## **5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

## **5.4 Further information**

Use water spray to cool unopened containers.

---

## **6. ACCIDENTAL RELEASE MEASURES**

### **6.1 Personal precautions, protective equipment and emergency procedures**

Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

For personal protection see section 8.

### **6.2 Environmental precautions**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

### **6.3 Methods and materials for containment and cleaning up**

Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13). Keep in suitable, closed containers for disposal.

### **6.4 Reference to other sections**

For disposal see section 13.

---

## **7. HANDLING AND STORAGE**

### **7.1 Precautions for safe handling**

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.

Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.

For precautions see section 2.2.

### **7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Light sensitive.

### **7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## **8. EXPOSURE CONTROLS/PERSONAL PROTECTION**

### **8.1 Control parameters**

#### **Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

### **8.2 Exposure controls**

#### **Appropriate engineering controls**

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

#### **Personal protective equipment**

##### **Eye/face protection**

Tightly fitting safety goggles. Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### **Skin protection**

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact

Material: butyl-rubber

Minimum layer thickness: 0.3 mm

Break through time: 480 min



Material tested:Butoject® (KCL 897 / Aldrich Z677647, Size M)

Splash contact

Material: Nature latex/chloroprene

Minimum layer thickness: 0.6 mm

Break through time: 48 min

Material tested:Lapren® (KCL 706 / Aldrich Z677558, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

### **Body Protection**

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

### **Respiratory protection**

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

### **Control of environmental exposure**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

## **9. PHYSICAL AND CHEMICAL PROPERTIES**

### **9.1 Information on basic physical and chemical properties**

- |   |  |
|---|--|
| a) Appearance                                   | Form: liquid   |
| b) Odour  | No data available                                    |
| c) Odour Threshold                              | No data available                                    |
| d) pH   | 7 - 10 at 200 g/l                                    |
| e) Melting point/freezing point                 | -39.99 °C (-39.98 °F)                                |
| f) Initial boiling point and boiling range      | 80 - 81 °C (176 - 178 °F) at 27 hPa (20 mmHg) - lit. |
| g) Flash point                                  | 70 °C (158 °F) - closed cup                          |
| h) Evaporation rate                             | No data available                                    |
| i) Flammability (solid, gas)                    | No data available                                    |
| j) Upper/lower flammability or explosive limits | Lower explosion limit: 0.68 %(V)                     |
| k) Vapour pressure                              | 0.65 hPa (0.49 mmHg) at 20 °C (68 °F)                |
| l) Vapour density                               | No data available                                    |
| m) Relative density                             | 0.962 g/cm <sup>3</sup> at 25 °C (77 °F)             |
| n) Water solubility                             | 1,000 g/l at 20 °C (68 °F)                           |
| o) Partition coefficient: n-octanol/water       | log Pow: -0.3 at 23 °C (73 °F)                       |
| p) Auto-ignition temperature                    | No data available                                    |
| q) Decomposition temperature                    | No data available                                    |
| r) Viscosity                                    | No data available                                    |

- s) Explosive properties      No data available
- t) Oxidizing properties      No data available

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

Contains the following stabiliser(s):

Mequinol (0.05 %)

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

Heat, flames and sparks.

### 10.5 Incompatible materials

Strong oxidizing agents, Strong bases, polymerisation initiators

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - > 215 - 464 mg/kg  
(OECD Test Guideline 401)

LD50 Dermal - Rabbit - 519 mg/kg

Remarks: Behavioral:Somnolence (general depressed activity). Behavioral:Tremor. Liver:Other changes.

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: Mild skin irritation

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Risk of serious damage to eyes.

(OECD Test Guideline 405)

Eyes - Rabbit

Result: Moderate eye irritation

#### Respiratory or skin sensitisation

- Guinea pig

Result: Did not cause sensitisation on laboratory animals.

(OECD Test Guideline 406)

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC:      No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH:      No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Stomach - Irregularities - Based on Human Evidence (Mequinol)

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish	LC50 - Oncorhynchus mykiss (rainbow trout) - > 120 mg/l - 96 h (OECD Test Guideline 203)
Toxicity to daphnia and other aquatic invertebrates	EC50 - Daphnia magna (Water flea) - > 120 mg/l - 48 h (OECD Test Guideline 202)
Toxicity to algae	EC50 - Pseudokirchneriella subcapitata (green algae) - > 400 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	EC50 - Bacteria - > 1,000 mg/l (OECD Test Guideline 209)

### 12.2 Persistence and degradability

Biodegradability Result: - Not readily biodegradable.  
(OECD Test Guideline 301C)

### 12.3 Bioaccumulative potential

No bioaccumulation is to be expected (log Pow <= 4).

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

**Product**

This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

**Contaminated packaging**

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

### DOT (US)

UN number: 2810      Class: 6.1      Packing group: III  
Proper shipping name: Toxic, liquids, organic, n.o.s. (N,N-Dimethylacrylamide)  
Reportable Quantity (RQ):  
Poison Inhalation Hazard: No

### IMDG

UN number: 2810      Class: 6.1      Packing group: III      EMS-No: F-A, S-A  
Proper shipping name: TOXIC LIQUID, ORGANIC, N.O.S. (N,N-Dimethylacrylamide)

### IATA

UN number: 2810      Class: 6.1      Packing group: III  
Proper shipping name: Toxic liquid, organic, n.o.s. (N,N-Dimethylacrylamide)

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Fire Hazard, Acute Health Hazard

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
N,N-Dimethylacrylamide	2680-03-7	

### New Jersey Right To Know Components

	CAS-No.	Revision Date
N,N-Dimethylacrylamide	2680-03-7	

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Dam.	Serious eye damage
Flam. Liq.	Flammable liquids
H227	Combustible liquid.
H301	Toxic if swallowed.
H301 + H311	Toxic if swallowed or in contact with skin
H311	Toxic in contact with skin.
H318	Causes serious eye damage.

### HMIS Rating

Health hazard:	2
Chronic Health Hazard:	
Flammability:	2
Physical Hazard	0

### NFPA Rating

Health hazard:	2
----------------	---

Fire Hazard: 2  
Reactivity Hazard: 0

**Further information**

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.5

Revision Date: 10/03/2017

Print Date: 04/07/2018

## SAFETY DATA SHEET

Version 5.4  
Revision Date 05/27/2016  
Print Date 04/10/2018

### 1. PRODUCT AND COMPANY IDENTIFICATION

#### 1.1 Product identifiers

Product name : Ethylenediamine

Product Number : E1649  
Brand : Sigma-Aldrich  
Index-No. : 612-006-00-6

CAS-No. : 107-15-3

#### 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

#### 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

#### 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

### 2. HAZARDS IDENTIFICATION

#### 2.1 Classification of the substance or mixture

##### GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Flammable liquids (Category 3), H226  
Acute toxicity, Oral (Category 4), H302  
Acute toxicity, Inhalation (Category 4), H332  
Acute toxicity, Dermal (Category 3), H311  
Skin corrosion (Category 1B), H314  
Serious eye damage (Category 1), H318  
Respiratory sensitisation (Category 1), H334  
Skin sensitisation (Category 1), H317  
Acute aquatic toxicity (Category 2), H401  
Chronic aquatic toxicity (Category 3), H412

For the full text of the H-Statements mentioned in this Section, see Section 16.

#### 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word : Danger

Hazard statement(s)

H226 : Flammable liquid and vapour.  
H302 + H332 : Harmful if swallowed or if inhaled  
H311 : Toxic in contact with skin.  
H314 : Causes severe skin burns and eye damage.

H317	May cause an allergic skin reaction.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H401	Toxic to aquatic life.
H412	Harmful to aquatic life with long lasting effects.
Precautionary statement(s)	
P210	Keep away from heat/sparks/open flames/hot surfaces. No smoking.
P233	Keep container tightly closed.
P240	Ground/bond container and receiving equipment.
P241	Use explosion-proof electrical/ ventilating/ lighting/ equipment.
P242	Use only non-sparking tools.
P243	Take precautionary measures against static discharge.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P271	Use only outdoors or in a well-ventilated area.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P285	In case of inadequate ventilation wear respiratory protection.
P301 + P312	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor.
P322	Specific measures (see supplemental first aid instructions on this label).
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
P361	Remove/Take off immediately all contaminated clothing.
P363	Wash contaminated clothing before reuse.
P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction.
P403 + P235	Store in a well-ventilated place. Keep cool.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS

Lachrymator.  
Rapidly absorbed through skin.

## 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.1 Substances

Synonyms	: 1,2-Diaminoethane
Formula	: C <sub>2</sub> H <sub>8</sub> N <sub>2</sub>
Molecular weight	: 60.10 g/mol
CAS-No.	: 107-15-3
EC-No.	: 203-468-6
Index-No.	: 612-006-00-6

#### Hazardous components

Component	Classification	Concentration
<b>Ethylenediamine</b>		
	Flam. Liq. 3; Acute Tox. 4; Acute Tox. 3; Skin Corr. 1B; Eye Dam. 1; Resp. Sens. 1;	<= 100 %

	Skin Sens. 1; Aquatic Acute 2; Aquatic Chronic 3; H226, H302 + H332, H311, H314, H317, H318, H334, H401, H412	
--	---	--

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

#### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital.

#### If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

Use water spray to cool unopened containers.

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas. For personal protection see section 8.

### 6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

### 6.3 Methods and materials for containment and cleaning up

Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).



## 6.4 Reference to other sections

For disposal see section 13.

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.  
Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.  
For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Air and moisture sensitive. Handle and store under inert gas.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Ethylenediamine	107-15-3	TWA	10.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
	Remarks	Not classifiable as a human carcinogen Danger of cutaneous absorption		
		TWA	10 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Not classifiable as a human carcinogen Danger of cutaneous absorption		
		TWA	10.000000 ppm 25.000000 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
		TWA	10 ppm 25 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
		TWA	10.000000 ppm 25.000000 mg/m3	USA. NIOSH Recommended Exposure Limits
		PEL	10 ppm 25 mg/m3	California permissible exposure limits for chemical contaminants (Title 8, Article 107)

### 8.2 Exposure controls

#### Appropriate engineering controls

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

#### Personal protective equipment

##### Eye/face protection

Tightly fitting safety goggles. Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact

Material: butyl-rubber  
Minimum layer thickness: 0.3 mm  
Break through time: 480 min  
Material tested: Butoject® (KCL 897 / Aldrich Z677647, Size M)

Splash contact  
Material: Nature latex/chloroprene  
Minimum layer thickness: 0.6 mm  
Break through time: 72 min  
Material tested: Lapren® (KCL 706 / Aldrich Z677558, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

### Body Protection

Complete suit protecting against chemicals, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |   |   |
|---|---|
| a) Appearance                                   | Form: liquid  |
| b) Odour  | No data available   |
| c) Odour Threshold                              | No data available   |
| d) pH   | 12.2 at 110 g/l at 20 °C (68 °F)                                  |
| e) Melting point/freezing point                 | Melting point/range: 8.5 °C (47.3 °F) - lit.                      |
| f) Initial boiling point and boiling range      | 118 °C (244 °F) - lit.  |
| g) Flash point                                  | 38 °C (100 °F) - closed cup                                       |
| h) Evaporation rate                             | No data available   |
| i) Flammability (solid, gas)                    | No data available   |
| j) Upper/lower flammability or explosive limits | Upper explosion limit: 16 %(V)<br>Lower explosion limit: 2.7 %(V) |
| k) Vapour pressure                              | 13 hPa (10 mmHg) at 20 °C (68 °F)                                 |
| l) Vapour density                               | 2.07 - (Air = 1.0)  |
| m) Relative density                             | 0.899 g/cm <sup>3</sup> at 25 °C (77 °F)                          |
| n) Water solubility                             | soluble   |
| o) Partition coefficient: n-octanol/water       | log Pow: -2.04  |

p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

## 9.2 Other safety information

Relative vapour density 2.07 - (Air = 1.0)

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

Heat, flames and sparks.

### 10.5 Incompatible materials

Oxidizing agents, Phosphorus halides, Aldehydes, Organic halides

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 1,200 mg/kg

Remarks: Behavioral:Ataxia.

LC50 Inhalation - Rat - 4 h - 14.7 mg/l

LD50 Dermal Dermal - Rabbit - 560 mg/kg

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: Causes burns.

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Corrosive

#### Respiratory or skin sensitisation

Maximisation Test - Guinea pig

Result: Causes sensitisation.

May cause allergic respiratory and skin reactions

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

Carcinogenicity - This product is or contains a component that is not classifiable as to its carcinogenicity based on its IARC, ACGIH, NTP, or EPA classification.

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: KH8575000

Vomiting, Diarrhoea, Abdominal pain, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Liver - Irregularities - Based on Human Evidence

Liver - Irregularities - Based on Human Evidence

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish	LC50 - Pimephales promelas (fathead minnow) - 115.7 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	EC50 - Daphnia magna (Water flea) - 3 mg/l - 48 h
	NOEC - Daphnia magna (Water flea) - 0.16 mg/l - 21 d
Toxicity to algae	EC50 - Pseudokirchneriella subcapitata (green algae) - 151 mg/l - 96 h

### 12.2 Persistence and degradability

Biodegradability	Biotic/Aerobic - Exposure time 28 d
	Result: 94 % - Readily biodegradable

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.  
Toxic to aquatic life.

Avoid release to the environment.

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

### DOT (US)

UN number: 1604      Class: 8 (3)      Packing group: II  
Proper shipping name: Ethylenediamine  
Reportable Quantity (RQ): 5000 lbs

Poison Inhalation Hazard: No

### IMDG

UN number: 1604      Class: 8 (3)      Packing group: II      EMS-No: F-E, S-C  
Proper shipping name: ETHYLENEDIAMINE

### IATA

UN number: 1604      Class: 8 (3)      Packing group: II  
Proper shipping name: Ethylenediamine

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

The following components are subject to reporting levels established by SARA Title III, Section 302:

	CAS-No.	Revision Date
Ethylenediamine	107-15-3	2007-03-01

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Fire Hazard, Acute Health Hazard, Chronic Health Hazard

### Massachusetts Right To Know Components

	CAS-No.	Revision Date
Ethylenediamine	107-15-3	2007-03-01

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Ethylenediamine	107-15-3	2007-03-01

### New Jersey Right To Know Components

	CAS-No.	Revision Date
Ethylenediamine	107-15-3	2007-03-01

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Aquatic Acute	Acute aquatic toxicity
Aquatic Chronic	Chronic aquatic toxicity
Eye Dam.	Serious eye damage
Flam. Liq.	Flammable liquids
H226	Flammable liquid and vapour.
H302	Harmful if swallowed.
H302 + H332	Harmful if swallowed or if inhaled
H311	Toxic in contact with skin.
H314	Causes severe skin burns and eye damage.
H317	May cause an allergic skin reaction.
H318	Causes serious eye damage.
H332	Harmful if inhaled.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H401	Toxic to aquatic life.
H412	Harmful to aquatic life with long lasting effects.

#### HMIS Rating

Health hazard:	3
Chronic Health Hazard:	*
Flammability:	2
Physical Hazard	0

#### NFPA Rating

Health hazard:	3
Fire Hazard:	2
Reactivity Hazard:	0

#### Further information

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

#### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.4

Revision Date: 05/27/2016

Print Date: 04/10/2018

## SAFETY DATA SHEET

Version 4.13  
Revision Date 10/03/2017  
Print Date 04/08/2018

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**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : Ethyl alcohol, pure

Product Number : E7023  
Brand : Sigma-Aldrich  
Index-No. : 603-002-00-5

CAS-No. : 64-17-5

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

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**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture****GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Flammable liquids (Category 2), H225  
Eye irritation (Category 2A), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

**2.2 GHS Label elements, including precautionary statements**

Pictogram



Signal word

Danger

Hazard statement(s)

H225 Highly flammable liquid and vapour.  
H319 Causes serious eye irritation.

Precautionary statement(s)

P210 Keep away from heat/sparks/open flames/hot surfaces. No smoking.  
P233 Keep container tightly closed.  
P240 Ground/bond container and receiving equipment.  
P241 Use explosion-proof electrical/ ventilating/ lighting/ equipment.  
P242 Use only non-sparking tools.  
P243 Take precautionary measures against static discharge.  
P264 Wash skin thoroughly after handling.  
P280 Wear protective gloves/ eye protection/ face protection.  
P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing.

P305 + P351 + P338	Rinse skin with water/shower.
P337 + P313	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P370 + P378	If eye irritation persists: Get medical advice/ attention.
P403 + P235	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
P501	Store in a well-ventilated place. Keep cool.
	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

### 3. COMPOSITION/INFORMATION ON INGREDIENTS

#### 3.1 Substances

Synonyms	: Absolute alcohol
Formula	: C <sub>2</sub> H <sub>6</sub> O
Molecular weight	: 46.07 g/mol
CAS-No.	: 64-17-5
EC-No.	: 200-578-6
Index-No.	: 603-002-00-5

#### Hazardous components

Component	Classification	Concentration
<b>Ethanol</b>		
	Flam. Liq. 2; Eye Irrit. 2A; H225, H319	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

### 4. FIRST AID MEASURES

#### 4.1 Description of first aid measures

##### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

##### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

##### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

##### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

##### If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

#### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

#### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

### 5. FIREFIGHTING MEASURES

#### 5.1 Extinguishing media

##### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.



## 5.2 Special hazards arising from the substance or mixture

No data available

## 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

## 5.4 Further information

Use water spray to cool unopened containers.

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

For personal protection see section 8.

### 6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.

Use explosion-proof equipment. Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Hygroscopic.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Ethanol	64-17-5	TWA	1,000.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
	Remarks	Upper Respiratory Tract irritation Confirmed animal carcinogen with unknown relevance to humans		
		TWA	1,000 ppm 1,900 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		TWA	1,000 ppm 1,900 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		

		TWA	1,000.000000 ppm 1,900.000000 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
		TWA	1,000.000000 ppm 1,900.000000 mg/m3	USA. NIOSH Recommended Exposure Limits
		STEL	1,000.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Upper Respiratory Tract irritation Confirmed animal carcinogen with unknown relevance to humans		

## 8.2 Exposure controls

### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

### Personal protective equipment

#### Eye/face protection

Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

#### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

#### Full contact

Material: butyl-rubber

Minimum layer thickness: 0.3 mm

Break through time: 480 min

Material tested: Butoject® (KCL 897 / Aldrich Z677647, Size M)

#### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.2 mm

Break through time: 38 min

Material tested: Dermatrill® P (KCL 743 / Aldrich Z677388, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

#### Body Protection

Impervious clothing, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

#### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

a) Appearance	Form: liquid, clear Colour: colourless
b) Odour	No data available
c) Odour Threshold	No data available
d) pH	No data available
e) Melting point/freezing point	-143.99 °C (-227.18 °F)
f) Initial boiling point and boiling range	78.0 - 80.0 °C (172.4 - 176.0 °F)
g) Flash point	14.0 °C (57.2 °F) - closed cup
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	Upper explosion limit: 19 %(V) Lower explosion limit: 3.3 %(V)
k) Vapour pressure	59.5 hPa (44.6 mmHg) at 20.0 °C (68.0 °F)
l) Vapour density	No data available
m) Relative density	0.7974 g/cm <sup>3</sup>
n) Water solubility	completely soluble
o) Partition coefficient: n-octanol/water	log Pow: -0.349 at 24 °C (75 °F)
p) Auto-ignition temperature	363.0 °C (685.4 °F)
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

### 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

Vapours may form explosive mixture with air.

### 10.4 Conditions to avoid

Heat, flames and sparks.

### 10.5 Incompatible materials

Alkali metals, Oxidizing agents, Peroxides

### 10.6 Hazardous decomposition products

Other decomposition products - No data available

Hazardous decomposition products formed under fire conditions. - Carbon oxides

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 10,470 mg/kg

LC50 Inhalation - Rat - 4 h - 30,000 mg/l

LD50 Dermal - Rabbit - 15,800 mg/kg

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: No skin irritation - 24 h

(OECD Test Guideline 404)

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Moderate eye irritation

(OECD Test Guideline 405)

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

Carcinogenicity - Mouse - Oral

Tumorigenic: Equivocal tumorigenic agent by RTECS criteria. Liver: Tumors. Blood: Lymphomas including Hodgkin's disease.

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

#### Reproductive toxicity

No data available

Reproductive toxicity - Human - female - Oral

Effects on Newborn: Apgar score (human only). Effects on Newborn: Other neonatal measures or effects. Effects on Newborn: Drug dependence.

#### Specific target organ toxicity - single exposure

No data available

#### Specific target organ toxicity - repeated exposure

No data available

#### Aspiration hazard

No data available

#### Additional Information

RTECS: KQ6300000

Central nervous system depression, narcosis, Damage to the heart., To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Stomach - Irregularities - Based on Human Evidence

Stomach - Irregularities - Based on Human Evidence

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish	LC50 - Pimephales promelas (fathead minnow) - 14,200 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	LC50 - Ceriodaphnia dubia (water flea) - 5,012 mg/l - 48 h
	NOEC - Daphnia magna (Water flea) - 9.6 mg/l - 9 d
Toxicity to algae	EC50 - Chlorella vulgaris (Fresh water algae) - 275 mg/l - 72 h (OECD Test Guideline 201)

### 12.2 Persistence and degradability

Biodegradability Result: 95 % - Readily biodegradable.

### 12.3 Bioaccumulative potential

Due to the distribution coefficient n-octanol/water, accumulation in organisms is not expected.

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

#### DOT (US)

UN number: 1170	Class: 3	Packing group: II
Proper shipping name: Ethanol		
Reportable Quantity (RQ):		
Poison Inhalation Hazard: No		

#### IMDG

UN number: 1170	Class: 3	Packing group: II	EMS-No: F-E, S-D
Proper shipping name: ETHANOL			

#### IATA

UN number: 1170	Class: 3	Packing group: II
Proper shipping name: Ethanol		

---

## 15. REGULATORY INFORMATION

#### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

#### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

Fire Hazard, Acute Health Hazard, Chronic Health Hazard

**Massachusetts Right To Know Components**

Ethanol

CAS-No.  
64-17-5Revision Date  
2007-03-01**Pennsylvania Right To Know Components**

Ethanol

CAS-No.  
64-17-5Revision Date  
2007-03-01**New Jersey Right To Know Components**

Ethanol

CAS-No.  
64-17-5Revision Date  
2007-03-01**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

**16. OTHER INFORMATION****Full text of H-Statements referred to under sections 2 and 3.**

Eye Irrit.	Eye irritation
Flam. Liq.	Flammable liquids
H225	Highly flammable liquid and vapour.
H319	Causes serious eye irritation.

**HMIS Rating**

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	3
Physical Hazard	0

**NFPA Rating**

Health hazard:	2
Fire Hazard:	3
Reactivity Hazard:	0

**Further information**

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 4.13

Revision Date: 10/03/2017

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 3.14  
Revision Date 02/24/2017  
Print Date 04/08/2018

### 1. PRODUCT AND COMPANY IDENTIFICATION

#### 1.1 Product identifiers

Product name : Glutaraldehyde solution

Product Number : G7776

Brand : Sigma-Aldrich

#### 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

#### 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

#### 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

### 2. HAZARDS IDENTIFICATION

#### 2.1 Classification of the substance or mixture

##### GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Acute toxicity, Oral (Category 3), H301

Acute toxicity, Inhalation (Category 3), H331

Skin corrosion (Category 1B), H314

Serious eye damage (Category 1), H318

Respiratory sensitisation (Category 1), H334

Skin sensitisation (Category 1), H317

Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335

Acute aquatic toxicity (Category 1), H400

Chronic aquatic toxicity (Category 2), H411

For the full text of the H-Statements mentioned in this Section, see Section 16.

#### 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Danger

Hazard statement(s)

H301 + H331

Toxic if swallowed or if inhaled

H314

Causes severe skin burns and eye damage.

H317

May cause an allergic skin reaction.

H334

May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H335

May cause respiratory irritation.

H400

Very toxic to aquatic life.

H411

Toxic to aquatic life with long lasting effects.

Precautionary statement(s)	
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P271	Use only outdoors or in a well-ventilated area.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P285	In case of inadequate ventilation wear respiratory protection.
P301 + P310 + P330	IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
P363	Wash contaminated clothing before reuse.
P391	Collect spillage.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

### 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

## 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.2 Mixtures

Synonyms : Pentane-1,5-dial  
Glutaric dialdehydesolution

Formula :  $C_5H_8O_2$   
Molecular weight : 100.12 g/mol

#### Hazardous components

Component		Classification	Concentration
<b>Glutaral</b>			
CAS-No.	111-30-8	Flam. Liq. 4; Acute Tox. 3; Acute Tox. 2; Skin Corr. 1B; Eye Dam. 1; Resp. Sens. 1; Skin Sens. 1; STOT SE 3; Aquatic Acute 1; Aquatic Chronic 2; H227, H301, H314, H317, H330, H334, H335, H400, H411	70 - 90 %
EC-No.	203-856-5		
Index-No.	605-022-00-X		
<b>Methanol</b>			
CAS-No.	67-56-1	Flam. Liq. 2; Acute Tox. 3; STOT SE 1; H225, H301 + H311 + H331, H370	0.1 - 1 %
EC-No.	200-659-6		
Index-No.	603-001-00-X		
Registration number	01-2119433307-44-XXXX		

For the full text of the H-Statements mentioned in this Section, see Section 16.



---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Move out of dangerous area. Consult a physician. Show this safety data sheet to the doctor in attendance.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

#### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital.

#### If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.

For personal protection see section 8.

### 6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

### 6.3 Methods and materials for containment and cleaning up

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.

For precautions see section 2.2.

## 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended storage temperature -20 °C

Store under inert gas. Air sensitive.

## 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

# 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

## 8.1 Control parameters

### Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Glutaral	111-30-8	C	0.200000 ppm 0.800000 mg/m3	USA. NIOSH Recommended Exposure Limits
	Remarks	See Appendix C		
		C	0.050000 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Central Nervous System impairment Upper Respiratory Tract irritation Eye irritation Skin irritation Not classifiable as a human carcinogen Sensitizer		
		C	0.05 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Dermal Sensitization Respiratory sensitization Central Nervous System impairment Upper Respiratory Tract irritation Eye irritation Skin irritation Not classifiable as a human carcinogen		
		C	0.05 ppm 0.2 mg/m3	California permissible exposure limits for chemical contaminants (Title 8, Article 107)
Methanol	67-56-1	TWA	200.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Headache Nausea Dizziness Eye damage Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Danger of cutaneous absorption		
		STEL	250.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Headache Nausea Dizziness Eye damage Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Danger of cutaneous absorption		

		TWA	200.000000 ppm 260.000000 mg/m3	USA. NIOSH Recommended Exposure Limits
		Potential for dermal absorption		
		ST	250.000000 ppm 325.000000 mg/m3	USA. NIOSH Recommended Exposure Limits
		Potential for dermal absorption		
		TWA	200.000000 ppm 260.000000 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
		TWA	200 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Headache Nausea Dizziness Eye damage Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Danger of cutaneous absorption		
		STEL	250 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Headache Nausea Dizziness Eye damage Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Danger of cutaneous absorption		
		TWA	200 ppm 260 mg/m3	USA. NIOSH Recommended Exposure Limits
		Potential for dermal absorption		
		ST	250 ppm 325 mg/m3	USA. NIOSH Recommended Exposure Limits
		Potential for dermal absorption		
		TWA	200 ppm 260 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
		STEL	250 ppm 325 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		Skin notation		
		TWA	200 ppm 260 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		Skin notation		
		C	1,000 ppm	California permissible exposure limits for chemical contaminants (Title 8, Article 107)
		Skin		
		PEL	200 ppm 260 mg/m3	California permissible exposure limits for chemical contaminants (Title 8, Article 107)
		Skin		

		STEL	250 ppm 325 mg/m3	California permissible exposure limits for chemical contaminants (Title 8, Article 107)
		Skin		

#### Biological occupational exposure limits

Component	CAS-No.	Parameters	Value	Biological specimen	Basis
Methanol	67-56-1	Methanol	15.0000 mg/l	Urine	ACGIH - Biological Exposure Indices (BEI)
	Remarks	End of shift (As soon as possible after exposure ceases)			
		Methanol	15 mg/l	Urine	ACGIH - Biological Exposure Indices (BEI)
		End of shift (As soon as possible after exposure ceases)			

## 8.2 Exposure controls

### Appropriate engineering controls

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

### Personal protective equipment

#### Eye/face protection

Tightly fitting safety goggles. Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

#### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

#### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.4 mm

Break through time: 480 min

Material tested: Camatril® (KCL 730 / Aldrich Z677442, Size M)

#### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 30 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

#### Body Protection

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

#### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

a) Appearance	Form: clear, liquid Colour: colourless
b) Odour	No data available
c) Odour Threshold	No data available
d) pH	No data available
e) Melting point/freezing point	No data available
f) Initial boiling point and boiling range	101 °C (214 °F) at 1,013 hPa (760 mmHg)
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	20 hPa (15 mmHg) at 20 °C (68 °F)
l) Vapour density	No data available
m) Relative density	1.143 g/cm <sup>3</sup>
n) Water solubility	soluble
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

### 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong bases, Acids, Oxidizing agents, Alkali metals, Strong oxidizing agents, Strong acids, Acid chlorides, Acid anhydrides, Reducing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides

Other decomposition products - No data available  
In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### **Acute toxicity**

No data available

Inhalation: No data available

Dermal: No data available

No data available

#### **Skin corrosion/irritation**

No data available

#### **Serious eye damage/eye irritation**

No data available

#### **Respiratory or skin sensitisation**

No data available

#### **Germ cell mutagenicity**

No data available

#### **Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

#### **Reproductive toxicity**

No data available

No data available

#### **Specific target organ toxicity - single exposure**

No data available

#### **Specific target organ toxicity - repeated exposure**

No data available

#### **Aspiration hazard**

No data available

#### **Additional Information**

RTECS: Not available

Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin., spasm, inflammation and edema of the larynx, spasm, inflammation and edema of the bronchi, pneumonitis, pulmonary edema, burning sensation, Cough, wheezing, laryngitis, Shortness of breath, Headache, Nausea

Liver - Irregularities - Based on Human Evidence

Liver - Irregularities - Based on Human Evidence (Glutaral)

Stomach - Irregularities - Based on Human Evidence (Methanol)

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

No data available

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Very toxic to aquatic life.

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

#### DOT (US)

UN number: 2922      Class: 8 (6.1)      Packing group: II

Proper shipping name: Corrosive liquids, toxic, n.o.s. (Glutaral)

Reportable Quantity (RQ):

Poison Inhalation Hazard: No

#### IMDG

UN number: 2922      Class: 8 (6.1)      Packing group: II      EMS-No: F-A, S-B

Proper shipping name: CORROSIVE LIQUID, TOXIC, N.O.S. (Glutaral)

Marine pollutant:yes

#### IATA

UN number: 2922      Class: 8 (6.1)      Packing group: II

Proper shipping name: Corrosive liquid, toxic, n.o.s. (Glutaral)

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Acute Health Hazard, Chronic Health Hazard

### Massachusetts Right To Know Components

Glutaral

CAS-No.  
111-30-8

Revision Date  
1993-04-24

### Pennsylvania Right To Know Components

Glutaral

CAS-No.  
111-30-8

Revision Date  
1993-04-24

Water

7732-18-5

Methanol

67-56-1

2007-07-01

### New Jersey Right To Know Components

Glutaral

CAS-No.  
111-30-8

Revision Date  
1993-04-24

Water

7732-18-5

Methanol

67-56-1

2007-07-01

### California Prop. 65 Components

WARNING: This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.

Methanol

CAS-No.  
67-56-1

Revision Date  
2012-03-16

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Aquatic Acute	Acute aquatic toxicity
Aquatic Chronic	Chronic aquatic toxicity
Eye Dam.	Serious eye damage
Flam. Liq.	Flammable liquids
H225	Highly flammable liquid and vapour.
H227	Combustible liquid.
H301	Toxic if swallowed.
H301 + H311 + H331	Toxic if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye damage.
H317	May cause an allergic skin reaction.
H318	Causes serious eye damage.
H330	Fatal if inhaled.
H331	Toxic if inhaled.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
H370	Causes damage to organs.
H400	Very toxic to aquatic life.
H411	Toxic to aquatic life with long lasting effects.
Resp. Sens.	Respiratory sensitisation
Skin Corr.	Skin corrosion
Skin Sens.	Skin sensitisation
STOT SE	Specific target organ toxicity - single exposure

### HMIS Rating

Health hazard:	4
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	1

### NFPA Rating

Health hazard:	3
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956





## SAFETY DATA SHEET

Version 5.4

Revision Date 08/31/2017

Print Date 04/10/2018

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1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Guanidine hydrochloride

Product Number : PHG0006

Brand : Sigma

Index-No. : 607-148-00-0

CAS-No. : 50-01-1

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Acute toxicity, Oral (Category 4), H302

Acute toxicity, Inhalation (Category 4), H332

Skin irritation (Category 2), H315

Eye irritation (Category 2A), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Warning

Hazard statement(s)

H302 + H332

H315

H319

Harmful if swallowed or if inhaled

Causes skin irritation.

Causes serious eye irritation.

Precautionary statement(s)

P261

P264

P270

P271

P280

P301 + P312 + P330

Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

Wash skin thoroughly after handling.

Do not eat, drink or smoke when using this product.

Use only outdoors or in a well-ventilated area.

Wear protective gloves/ eye protection/ face protection.

IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

P302 + P352	Rinse mouth.
P304 + P340 + P312	IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
P362	Take off contaminated clothing and wash before reuse.
P501	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

## 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.1 Substances

Formula	: CH <sub>5</sub> N <sub>3</sub> · HCl
Molecular weight	: 95.53 g/mol
CAS-No.	: 50-01-1
EC-No.	: 200-002-3
Index-No.	: 607-148-00-0

#### Hazardous components

Component	Classification	Concentration
<b>Guanidinium chloride</b>		
	Acute Tox. 4; Skin Irrit. 2; Eye Irrit. 2A; H302 + H332, H315, H319	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

#### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

#### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

## **5.2 Special hazards arising from the substance or mixture**

No data available

## **5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

## **5.4 Further information**

No data available

---

# **6. ACCIDENTAL RELEASE MEASURES**

## **6.1 Personal precautions, protective equipment and emergency procedures**

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

For personal protection see section 8.

## **6.2 Environmental precautions**

Do not let product enter drains.

## **6.3 Methods and materials for containment and cleaning up**

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

## **6.4 Reference to other sections**

For disposal see section 13.

---

# **7. HANDLING AND STORAGE**

## **7.1 Precautions for safe handling**

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

## **7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

## **7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

# **8. EXPOSURE CONTROLS/PERSONAL PROTECTION**

## **8.1 Control parameters**

### **Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

Hazardous components without workplace control parameters

## **8.2 Exposure controls**

### **Appropriate engineering controls**

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

### **Personal protective equipment**

#### **Eye/face protection**

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

#### **Skin protection**

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

#### **Body Protection**

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

**Respiratory protection**

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**Control of environmental exposure**

Do not let product enter drains.

---

**9. PHYSICAL AND CHEMICAL PROPERTIES****9.1 Information on basic physical and chemical properties**

a) Appearance	Form: crystalline Colour: white
b) Odour	No data available
c) Odour Threshold	No data available
d) pH	4.5 - 6 at 573 g/l at 25 °C (77 °F)
e) Melting point/freezing point	185 - 189 °C (365 - 372 °F)
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	1.345 g/cm <sup>3</sup> at 20 °C (68 °F)
n) Water solubility	573 g/l at 20 °C (68 °F) - completely soluble
o) Partition coefficient: n-octanol/water	log Pow: -1.7 at ca. 20 °C (68 °F)
p) Auto-ignition temperature	No data available
q) Decomposition temperature	> 310 °C (> 590 °F) -
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

**9.2 Other safety information**

No data available

---

**10. STABILITY AND REACTIVITY****10.1 Reactivity**

No data available

**10.2 Chemical stability**

Stable under recommended storage conditions.

**10.3 Possibility of hazardous reactions**

No data available

#### 10.4 Conditions to avoid

No data available

#### 10.5 Incompatible materials

Strong oxidizing agents

#### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NO<sub>x</sub>), Hydrogen chloride gas

Other decomposition products - No data available

In the event of fire: see section 5

---

### 11. TOXICOLOGICAL INFORMATION

#### 11.1 Information on toxicological effects

##### Acute toxicity

LD50 Oral - Rat - 475 mg/kg

Remarks: Behavioral:Altered sleep time (including change in righting reflex). Behavioral:Excitement. Diarrhoea

LD50 Oral - Mouse - 571 mg/kg

Remarks: Behavioral:Altered sleep time (including change in righting reflex). Behavioral:Muscle contraction or spasticity. Behavioral:Irritability.

LD50 Oral - Rat - 1,120 mg/kg

LC50 Inhalation - Rat - female - 4 h - 3.181 mg/l  
(OECD Test Guideline 403)

Dermal: No data available

No data available

##### Skin corrosion/irritation

Skin - Rabbit

Result: Skin irritation

##### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Irritating to eyes.

##### Respiratory or skin sensitisation

Buehler Test - Guinea pig

Did not cause sensitisation on laboratory animals.

##### Germ cell mutagenicity

Not mutagenic in Ames Test

##### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

##### Reproductive toxicity

No data available

No data available

##### Specific target organ toxicity - single exposure

No data available

##### Specific target organ toxicity - repeated exposure

No data available

##### Aspiration hazard

No data available

**Additional Information**

RTECS: MF4300000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Liver - Irregularities - Based on Human Evidence

Liver - Irregularities - Based on Human Evidence

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

Toxicity to fish                      LC50 - Leuciscus idus (Golden orfe) - 1,759 mg/l

**12.2 Persistence and degradability**

Biodegradability                      Result: - Not readily biodegradable.

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION****DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION****SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

Acute Health Hazard, Chronic Health Hazard

**Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

**Pennsylvania Right To Know Components**

Guanidinium chloride

CAS-No.  
50-01-1

Revision Date

Guanidinium chloride

CAS-No.  
50-01-1

Revision Date

#### New Jersey Right To Know Components

Guanidinium chloride

CAS-No.  
50-01-1

Revision Date

#### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Irrit.	Eye irritation
H302	Harmful if swallowed.
H302 + H332	Harmful if swallowed or if inhaled
H315	Causes skin irritation.
H319	Causes serious eye irritation.

### HMIS Rating

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	2
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.4

Revision Date: 08/31/2017

Print Date: 04/10/2018



## SAFETY DATA SHEET

Version 5.8  
Revision Date 08/31/2017  
Print Date 04/10/2018

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : HEPES

Product Number : RES6003H-B7

Brand : Sigma

CAS-No. : 7365-45-9

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture.

**2.2 GHS Label elements, including precautionary statements**

Not a hazardous substance or mixture.

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none**

---

**3. COMPOSITION/INFORMATION ON INGREDIENTS****3.1 Substances**

Formula :  $C_8H_{18}N_2O_4S$

Molecular weight : 238.3 g/mol

CAS-No. : 7365-45-9

EC-No. : 230-907-9

Registration number : 01-2120054645-54-XXXX

No components need to be disclosed according to the applicable regulations.

---

**4. FIRST AID MEASURES****4.1 Description of first aid measures****If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

**In case of skin contact**

Wash off with soap and plenty of water.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

**6.2 Environmental precautions**

No special environmental precautions required.

**6.3 Methods and materials for containment and cleaning up**

Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

**8. EXPOSURE CONTROLS/PERSONAL PROTECTION****8.1 Control parameters****Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

## 8.2 Exposure controls

### Appropriate engineering controls

General industrial hygiene practice.

### Personal protective equipment

#### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

#### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

#### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

#### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Control of environmental exposure

No special environmental precautions required.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |   |   |
|---|---|
| a) Appearance                                   | Form: Crystalline powder<br>Colour: colourless                        |
| b) Odour  | No data available   |
| c) Odour Threshold                              | No data available   |
| d) pH   | 5.0 - 6.5 at 238 g/l at 25 °C (77 °F)                                 |
| e) Melting point/freezing point                 | Melting point/range: 212.6 °C (414.7 °F) - Decomposes before melting. |
| f) Initial boiling point and boiling range      | Decomposes below the boiling point.                                   |
| g) Flash point                                  | No data available   |
| h) Evaporation rate                             | No data available   |
| i) Flammability (solid, gas)                    | The product is not flammable. - Flammability (solids)                 |
| j) Upper/lower flammability or explosive limits | No data available   |
| k) Vapour pressure                              | No data available   |
| l) Vapour density                               | No data available   |
| m) Relative density                             | 1.439 g/cm <sup>3</sup> at 20 °C (68 °F)                              |
| n) Water solubility                             | 703.6 g/l at 20 °C (68 °F) - OECD Test Guideline 105                  |
| o) Partition coefficient: n-octanol/water       | log Pow: < -3.85  |
| p) Auto-ignition temperature                    | does not ignite   |
| q) Decomposition temperature                    | No data available   |

- |                         |  |
|-------------------------|--|
| r) Viscosity            | No data available  |
| s) Explosive properties | Not explosive  |
| t) Oxidizing properties | The substance or mixture is not classified as oxidizing. |

## 9.2 Other safety information

Surface tension	63.98 mN/m at 20 °C (68 °F)
-----------------	-----------------------------

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Other decomposition products - No data available

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NO<sub>x</sub>), Sulphur oxides

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

No data available

LD50 Oral - Rat - > 2,000 mg/kg

Inhalation: No data available

Inhalation: No data available

Dermal: No data available

LD50 Dermal - Rat - > 2,000 mg/kg  
(OECD Test Guideline 402)

No data available

No data available

#### Skin corrosion/irritation

No data available

Skin - Rabbit

Result: No skin irritation

(OECD Test Guideline 404)

#### Serious eye damage/eye irritation

No data available

Eyes - Rabbit

Result: No eye irritation

(OECD Test Guideline 405)

#### Respiratory or skin sensitisation

No data available

Maximisation Test - Guinea pig

Did not cause sensitisation on laboratory animals.

**Germ cell mutagenicity**

No data available

in vitro assay

Result: negative

Lymphoma Mutation Assay

Chromosome aberration test in vitro

Result: negative

Result: Not mutagenic in Ames Test

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

Did not show teratogenic effects in animal experiments.

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

Repeated dose toxicity      Rat - Oral - 28 d - Subacute oral toxicity - NOAEL : 1,000 mg/kg - OECD Test Guideline 407

RTECS: TL6809000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

No data available

Toxicity to fish      LC50 - Brachydanio rerio (zebrafish) - > 100 mg/l - 96 h  
(OECD Test Guideline 203)

Toxicity to daphnia and other aquatic invertebrates      EC50 - Daphnia magna (Water flea) - > 100 mg/l - 48 h

Toxicity to algae      static test EC50 - Pseudokirchneriella subcapitata (green algae) - > 100 mg/l - 72 h  
(OECD Test Guideline 201)

NOEC - Pseudokirchneriella subcapitata (green algae) - 100 mg/l - 72 h  
(OECD Test Guideline 201)

Toxicity to bacteria      EC50 - Sludge Treatment - > 1,000 mg/l - 3 h  
(OECD Test Guideline 209)

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

No data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION****DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION****SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

No SARA Hazards

**Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

**Pennsylvania Right To Know Components**

4-(2-Hydroxyethyl)piperazin-1-ylethanesulphonic acid	CAS-No. 7365-45-9	Revision Date
--	----------------------	---------------

4-(2-Hydroxyethyl)piperazin-1-ylethanesulphonic acid	CAS-No. 7365-45-9	Revision Date
--	----------------------	---------------

**New Jersey Right To Know Components**

4-(2-Hydroxyethyl)piperazin-1-ylethanesulphonic acid	CAS-No. 7365-45-9	Revision Date
--	----------------------	---------------

**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### HMIS Rating

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.8

Revision Date: 08/31/2017

Print Date: 04/10/2018

## SAFETY DATA SHEET

Version 5.0

Revision Date 01/16/2018

Print Date 04/10/2018

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1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Imidazole (200 mg)

Product Number : 1336500

Brand : USP

CAS-No. : 288-32-4

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

## GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Acute toxicity, Oral (Category 4), H302

Skin corrosion (Category 1C), H314

Serious eye damage (Category 1), H318

Reproductive toxicity (Category 1B), H360D

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Danger

Hazard statement(s)

H302

Harmful if swallowed.

H314

Causes severe skin burns and eye damage.

H360D

May damage the unborn child.

Precautionary statement(s)

P201

Obtain special instructions before use.

P202

Do not handle until all safety precautions have been read and understood.

P260

Do not breathe dust or mist.

P264

Wash skin thoroughly after handling.

P270

Do not eat, drink or smoke when using this product.

P280

Wear protective gloves/ protective clothing/ eye protection/ face



P301 + P312 + P330	protection. IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.
P363	Wash contaminated clothing before reuse.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

### 3. COMPOSITION/INFORMATION ON INGREDIENTS

#### 3.1 Substances

Molecular weight	: 68.08 g/mol
CAS-No.	: 288-32-4

#### Hazardous components

Component	Classification	Concentration
<b>Imidazole</b>		
	Acute Tox. 4; Skin Corr. 1C; Eye Dam. 1; Repr. 1B; H302, H314, H360D	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

### 4. FIRST AID MEASURES

#### 4.1 Description of first aid measures

##### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

##### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

##### In case of skin contact

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician.

##### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital.

##### If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

#### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

#### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### **Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

For personal protection see section 8.

### 6.2 Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs. Avoid contact with skin and eyes. Avoid formation of dust and aerosols.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Storage class (TRGS 510): 6.1D: Non-combustible, acute toxic Cat.3 / toxic hazardous materials or hazardous materials causing chronic effects

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### **Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

Hazardous components without workplace control parameters

### 8.2 Exposure controls

#### **Appropriate engineering controls**

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### **Personal protective equipment**

##### **Eye/face protection**

Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

**Skin protection**

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

**Body Protection**

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

**Respiratory protection**

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**Control of environmental exposure**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

**9. PHYSICAL AND CHEMICAL PROPERTIES****9.1 Information on basic physical and chemical properties**

a) Appearance	Form: crystalline Colour: white
b) Odour	amine-like
c) Odour Threshold	No data available
d) pH	9 - 11 at 100 g/l at 23 °C (73 °F)
e) Melting point/freezing point	89 - 91 °C (192 - 196 °F)
f) Initial boiling point and boiling range	256 °C (493 °F) at 1,013 hPa (760 mmHg)
g) Flash point	145 °C (293 °F) - closed cup
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	0.003 hPa (0.002 mmHg) at 20 °C (68 °F)
l) Vapour density	No data available
m) Relative density	1.030 g/cm <sup>3</sup>
n) Water solubility	633 g/l at 20 °C (68 °F)
o) Partition coefficient: n-octanol/water	log Pow: -0.02 at 25 °C (77 °F)
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

**9.2 Other safety information**

Bulk density	550 kg/m <sup>3</sup>
Dissociation constant	7.15 at 25 °C (77 °F)

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

acids, Acid anhydrides, Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NO<sub>x</sub>), Hydrogen cyanide (hydrocyanic acid)

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 970 mg/kg

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: Corrosive, category 1C - where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

#### Serious eye damage/eye irritation

No data available

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

Did not show mutagenic effects in animal experiments. Tests on bacterial or mammalian cell cultures did not show mutagenic effects.

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

#### Reproductive toxicity

Presumed human reproductive toxicant May damage the unborn child.

No data available

#### Specific target organ toxicity - single exposure

No data available

#### Specific target organ toxicity - repeated exposure

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

Toxicity to fish	static test LC50 - Leuciscus idus (Golden orfe) - 280 mg/l - 48 h
Toxicity to daphnia and other aquatic invertebrates	EC50 - Daphnia (water flea) - 341.5 mg/l - 48 h
Toxicity to algae	static test EC50 - Scenedesmus quadricauda (Green algae) - 133 mg/l - 72 h
Toxicity to bacteria	see user defined free text - other microorganisms - 45 mg/l - 0.5 h

**12.2 Persistence and degradability**

Biodegradability	aerobic - Exposure time 19 d Result: 86 % - Readily biodegradable.
------------------	---

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION****DOT (US)**

UN number: 3263      Class: 8      Packing group: III  
Proper shipping name: Corrosive solid, basic, organic, n.o.s. (Imidazole)  
Reportable Quantity (RQ):  
Poison Inhalation Hazard: No

**IMDG**

UN number: 3263      Class: 8      Packing group: III      EMS-No: F-A, S-B  
Proper shipping name: CORROSIVE SOLID, BASIC, ORGANIC, N.O.S. (Imidazole)

**IATA**

UN number: 3263      Class: 8      Packing group: III  
Proper shipping name: Corrosive solid, basic, organic, n.o.s. (Imidazole)

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Acute Health Hazard, Chronic Health Hazard

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Imidazole	288-32-4	

	CAS-No.	Revision Date
Imidazole	288-32-4	

### New Jersey Right To Know Components

	CAS-No.	Revision Date
Imidazole	288-32-4	

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Dam.	Serious eye damage
H302	Harmful if swallowed.
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H360D	May damage the unborn child.
Repr.	Reproductive toxicity
Skin Corr.	Skin corrosion

### HMIS Rating

Health hazard:	3
Chronic Health Hazard:	*
Flammability:	1
Physical Hazard	0

### NFPA Rating

Health hazard:	3
Fire Hazard:	1
Reactivity Hazard:	0

### Further information

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.0

Revision Date: 01/16/2018

Print Date: 04/10/2018

## SAFETY DATA SHEET

Version 5.3  
Revision Date 03/04/2018  
Print Date 04/10/2018

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : Mannitol

Product Number : 1375105

Brand : USP

CAS-No. : 69-65-8

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture.

**2.2 GHS Label elements, including precautionary statements**

Not a hazardous substance or mixture.

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none**

---

**3. COMPOSITION/INFORMATION ON INGREDIENTS****3.1 Substances**

Synonyms : D-Mannitol  
Mannite

Formula : C<sub>6</sub>H<sub>14</sub>O<sub>6</sub>

Molecular weight : 182.17 g/mol

CAS-No. : 69-65-8

EC-No. : 200-711-8

No components need to be disclosed according to the applicable regulations.



---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### **If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

#### **In case of skin contact**

Wash off with soap and plenty of water.

#### **In case of eye contact**

Flush eyes with water as a precaution.

#### **If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### **Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

### 6.2 Environmental precautions

No special environmental precautions required.

### 6.3 Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Keep in a dry place.

Storage class (TRGS 510): 13: Non Combustible Solids

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

General industrial hygiene practice.

#### Personal protective equipment

##### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

No special environmental precautions required.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |   |   |
|---|---|
| a) Appearance                                   | Form: powder<br>Colour: white                 |
| b) Odour  | No data available                             |
| c) Odour Threshold                              | No data available                             |
| d) pH   | 5.0 - 6.5 at 182 g/l at 25 °C (77 °F)         |
| e) Melting point/freezing point                 | 165 °C (329 °F)                               |
| f) Initial boiling point and boiling range      | No data available                             |
| g) Flash point                                  | No data available                             |
| h) Evaporation rate                             | No data available                             |
| i) Flammability (solid, gas)                    | No data available                             |
| j) Upper/lower flammability or explosive limits | No data available                             |
| k) Vapour pressure                              | No data available                             |
| l) Vapour density                               | No data available                             |
| m) Relative density                             | No data available                             |
| n) Water solubility                             | 182 g/l at 20 °C (68 °F) - completely soluble |
| o) Partition coefficient: n-                    | No data available                             |

octanol/water

- |    |                           |                   |
|----|---------------------------|-------------------|
| p) | Auto-ignition temperature | No data available |
| q) | Decomposition temperature | No data available |
| r) | Viscosity                 | No data available |
| s) | Explosive properties      | No data available |
| t) | Oxidizing properties      | No data available |

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 13,500 mg/kg

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

No data available

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's

list of regulated carcinogens.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION**

**12.1 Toxicity**

No data available

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS**

**13.1 Waste treatment methods**

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION**

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION**

**SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

#### **SARA 311/312 Hazards**

No SARA Hazards

#### **Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

#### **Pennsylvania Right To Know Components**

	CAS-No.	Revision Date
D-Mannitol	69-65-8	

	CAS-No.	Revision Date
D-Mannitol	69-65-8	

#### **New Jersey Right To Know Components**

	CAS-No.	Revision Date
D-Mannitol	69-65-8	

#### **California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## **16. OTHER INFORMATION**

#### **HMIS Rating**

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

#### **NFPA Rating**

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

#### **Further information**

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#### **Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.3

Revision Date: 03/04/2018

Print Date: 04/10/2018

## SAFETY DATA SHEET

Version 5.6  
Revision Date 09/05/2017  
Print Date 04/07/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : *N,N'*-Methylenebis(acrylamide)

Product Number : 146072  
Brand : Sigma-Aldrich

CAS-No. : 110-26-9

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Acute toxicity, Oral (Category 4), H302

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word : Warning

Hazard statement(s)

H302 : Harmful if swallowed.

Precautionary statement(s)

P264 : Wash skin thoroughly after handling.  
P270 : Do not eat, drink or smoke when using this product.  
P301 + P312 + P330 : IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.  
Rinse mouth.  
P501 : Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Synonyms : Bis-acrylamide

Formula : C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>  
Molecular weight : 154.17 g/mol  
CAS-No. : 110-26-9  
EC-No. : 203-750-9

#### Hazardous components

Component	Classification	Concentration
<b>N,N'-Methylenediacrylamide</b>		
	Acute Tox. 4; H302	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

#### In case of eye contact

Flush eyes with water as a precaution.

#### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

For personal protection see section 8.

### 6.2 Environmental precautions

Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature 2 - 8 °C

Air and light sensitive.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

Hazardous components without workplace control parameters

### 8.2 Exposure controls

#### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### Personal protective equipment

##### Eye/face protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

##### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.



**Body Protection**

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

**Respiratory protection**

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**Control of environmental exposure**

Do not let product enter drains.

---

**9. PHYSICAL AND CHEMICAL PROPERTIES****9.1 Information on basic physical and chemical properties**

a) Appearance	Form: solid
b) Odour	No data available
c) Odour Threshold	No data available
d) pH	No data available
e) Melting point/freezing point	Melting point/range: > 300 °C (> 572 °F)
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	No data available
l) Vapour density	5.32 - (Air = 1.0)
m) Relative density	No data available
n) Water solubility	20 g/l at 20 °C (68 °F) - completely soluble
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

**9.2 Other safety information**

Relative vapour density	5.32 - (Air = 1.0)
-------------------------	--------------------

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**10. STABILITY AND REACTIVITY****10.1 Reactivity**

No data available

**10.2 Chemical stability**

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

Exposure to air.

### 10.5 Incompatible materials

Acids, Bases, Oxidizing agents, Reducing agents, Iron and iron salts., Copper, Aluminum, Brass, Free radical initiators

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 390 mg/kg

Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste):Eye:Other. Behavioral:Tremor. Lungs, Thorax, or Respiration:Other changes.

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

No data available

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

#### Reproductive toxicity

No data available

Overexposure may cause reproductive disorder(s) based on tests with laboratory animals.

#### Specific target organ toxicity - single exposure

No data available

#### Specific target organ toxicity - repeated exposure

No data available

#### Aspiration hazard

No data available

#### Additional Information

RTECS: AS3678000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish LC50 - Oncorhynchus mykiss (rainbow trout) - > 100 mg/l - 96 h

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

#### DOT (US)

Not dangerous goods

#### IMDG

Not dangerous goods

#### IATA

Not dangerous goods

---

## 15. REGULATORY INFORMATION

#### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

#### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

#### SARA 311/312 Hazards

Acute Health Hazard

#### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

#### Pennsylvania Right To Know Components

N,N'-Methylenediacrylamide	CAS-No. 110-26-9	Revision Date
----------------------------	---------------------	---------------

N,N'-Methylenediacrylamide	CAS-No. 110-26-9	Revision Date
----------------------------	---------------------	---------------

## New Jersey Right To Know Components

N,N'-Methylenediacrylamide

CAS-No.  
110-26-9

Revision Date

## California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
H302	Harmful if swallowed.

### HMIS Rating

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	1

### NFPA Rating

Health hazard:	2
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.6

Revision Date: 09/05/2017

Print Date: 04/07/2018

## SAFETY DATA SHEET

Version 4.5  
Revision Date 05/11/2016  
Print Date 04/08/2018

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : MES

Product Number : M3671

Brand : Sigma

CAS-No. : 4432-31-9

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture.

**2.2 GHS Label elements, including precautionary statements**

Not a hazardous substance or mixture.

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none**

---

**3. COMPOSITION/INFORMATION ON INGREDIENTS****3.1 Substances**

Synonyms : 4-Morpholineethanesulfonic acid  
2-(N-Morpholino)ethanesulfonic acid

Formula : C<sub>6</sub>H<sub>13</sub>NO<sub>4</sub>S

Molecular weight : 195.24 g/mol

CAS-No. : 4432-31-9

EC-No. : 224-632-3

No components need to be disclosed according to the applicable regulations.

---

**4. FIRST AID MEASURES****4.1 Description of first aid measures****If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

**In case of skin contact**

Wash off with soap and plenty of water.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

**6.2 Environmental precautions**

Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

Keep in a dry place.

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

**8. EXPOSURE CONTROLS/PERSONAL PROTECTION****8.1 Control parameters****Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

## 8.2 Exposure controls

### Appropriate engineering controls

General industrial hygiene practice.

### Personal protective equipment

#### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

#### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

#### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

#### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Control of environmental exposure

Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |  |                                      |
|--|--------------------------------------|
| a) Appearance                              | Form: powder<br>Colour: white        |
| b) Odour                                   | No data available                    |
| c) Odour Threshold                         | No data available                    |
| d) pH                                      | 2.5 - 4 at 97.6 g/l at 25 °C (77 °F) |
| e) Melting point/freezing point            | 300 °C (572 °F)                      |
| f) Initial boiling point and boiling range | No data available                    |
| g) Flash point                             | No data available                    |
| h) Evaporation rate                        | No data available                    |

- |    |  |  |
|----|--|--|
| i) | Flammability (solid, gas)                    | No data available                              |
| j) | Upper/lower flammability or explosive limits | No data available                              |
| k) | Vapour pressure                              | No data available                              |
| l) | Vapour density                               | No data available                              |
| m) | Relative density                             | No data available                              |
| n) | Water solubility                             | 97.6 g/l at 20 °C (68 °F) - completely soluble |
| o) | Partition coefficient: n-octanol/water       | No data available                              |
| p) | Auto-ignition temperature                    | No data available                              |
| q) | Decomposition temperature                    | No data available                              |
| r) | Viscosity                                    | No data available                              |
| s) | Explosive properties                         | No data available                              |
| t) | Oxidizing properties                         | No data available                              |

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx), Sulphur oxides

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

No data available

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

No data available



**Respiratory or skin sensitisation**

No data available

**Germ cell mutagenicity**

No data available

**Carcinogenicity**

- IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
- ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
- NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
- OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: QE3479500

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

No data available

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

### DOT (US)

Not dangerous goods

### IMDG

Not dangerous goods

### IATA

Not dangerous goods

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

No SARA Hazards

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
2-Morpholinoethanesulphonic acid	145224-94-8	

### New Jersey Right To Know Components

	CAS-No.	Revision Date
2-Morpholinoethanesulphonic acid	145224-94-8	

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### HMIS Rating

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 4.5

Revision Date: 05/11/2016

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 5.6  
Revision Date 08/31/2017  
Print Date 04/08/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Sodium phosphate monobasic monohydrate

Product Number : RES20906-A7

Brand : Sigma

CAS-No. : 10049-21-5

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

## 2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Synonyms : Monosodium phosphate  
Sodium dihydrogen phosphate monohydrate  
Sodium phosphate monobasic monohydrate

Formula :  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

Molecular weight : 137.99 g/mol

CAS-No. : 10049-21-5

EC-No. : 231-834-5

No components need to be disclosed according to the applicable regulations.

---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### **If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

#### **In case of skin contact**

Wash off with soap and plenty of water.

#### **In case of eye contact**

Flush eyes with water as a precaution.

#### **If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### **Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

### 6.2 Environmental precautions

No special environmental precautions required.

### 6.3 Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

General industrial hygiene practice.

#### Personal protective equipment

##### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

No special environmental precautions required.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |   |                                      |
|---|--------------------------------------|
| a) Appearance                                   | Form: crystalline<br>Colour: white   |
| b) Odour  | No data available                    |
| c) Odour Threshold                              | No data available                    |
| d) pH   | 4.1 - 4.5 at 50 g/l at 25 °C (77 °F) |
| e) Melting point/freezing point                 | No data available                    |
| f) Initial boiling point and boiling range      | No data available                    |
| g) Flash point                                  | No data available                    |
| h) Evaporation rate                             | No data available                    |
| i) Flammability (solid, gas)                    | No data available                    |
| j) Upper/lower flammability or explosive limits | No data available                    |
| k) Vapour pressure                              | No data available                    |
| l) Vapour density                               | No data available                    |
| m) Relative density                             | No data available                    |
| n) Water solubility                             | No data available                    |
| o) Partition coefficient: n-                    | No data available                    |

octanol/water

- |    |                           |                   |
|----|---------------------------|-------------------|
| p) | Auto-ignition temperature | No data available |
| q) | Decomposition temperature | No data available |
| r) | Viscosity                 | No data available |
| s) | Explosive properties      | No data available |
| t) | Oxidizing properties      | No data available |

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents, Strong acids

### 10.6 Hazardous decomposition products

Other decomposition products - No data available

Hazardous decomposition products formed under fire conditions. - Oxides of phosphorus, Sodium oxides

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

No data available

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

No data available

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a

carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION**

**12.1 Toxicity**

No data available

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS**

**13.1 Waste treatment methods**

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION**

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION**

**SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**



This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

#### **SARA 311/312 Hazards**

No SARA Hazards

#### **Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

#### **Pennsylvania Right To Know Components**

	CAS-No.	Revision Date
Sodium dihydrogenorthophosphate monohydrate	10049-21-5	

	CAS-No.	Revision Date
Sodium dihydrogenorthophosphate monohydrate	10049-21-5	

#### **New Jersey Right To Know Components**

	CAS-No.	Revision Date
Sodium dihydrogenorthophosphate monohydrate	10049-21-5	

#### **California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## **16. OTHER INFORMATION**

#### **HMIS Rating**

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

#### **NFPA Rating**

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

#### **Further information**

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#### **Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

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## SAFETY DATA SHEET

Version 4.10  
Revision Date 10/03/2017  
Print Date 04/07/2018

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : Sodium phosphate

Product Number : 342483

Brand : Aldrich

CAS-No. : 7601-54-9

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture****GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Skin irritation (Category 2), H315

Eye irritation (Category 2A), H319

Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335

For the full text of the H-Statements mentioned in this Section, see Section 16.

**2.2 GHS Label elements, including precautionary statements**

Pictogram



Signal word

Warning

Hazard statement(s)

H315

Causes skin irritation.

H319

Causes serious eye irritation.

H335

May cause respiratory irritation.

Precautionary statement(s)

P261

Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P264

Wash skin thoroughly after handling.

P271

Use only outdoors or in a well-ventilated area.

P280

Wear protective gloves/ eye protection/ face protection.

P302 + P352

IF ON SKIN: Wash with plenty of soap and water.

P304 + P340

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

P305 + P351 + P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove

P312	contact lenses, if present and easy to do. Continue rinsing.
P321	Call a POISON CENTER/doctor if you feel unwell.
P332 + P313	Specific treatment (see supplemental first aid instructions on this label).
P337 + P313	If skin irritation occurs: Get medical advice/ attention.
P362	If eye irritation persists: Get medical advice/ attention.
P403 + P233	Take off contaminated clothing and wash before reuse.
P405	Store in a well-ventilated place. Keep container tightly closed.
P501	Store locked up.
	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

### 3. COMPOSITION/INFORMATION ON INGREDIENTS

#### 3.1 Substances

Synonyms	: Trisodium phosphate
Formula	: Na <sub>3</sub> O <sub>4</sub> P
Molecular weight	: 163.94 g/mol
CAS-No.	: 7601-54-9
EC-No.	: 231-509-8

#### Hazardous components

Component	Classification	Concentration
<b>Trisodium orthophosphate</b>		
	Skin Irrit. 2; Eye Irrit. 2A; STOT SE 3; H315, H319, H335	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

### 4. FIRST AID MEASURES

#### 4.1 Description of first aid measures

##### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

##### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

##### In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

##### In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

##### If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

#### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

#### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

### 5. FIREFIGHTING MEASURES

#### 5.1 Extinguishing media

##### Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

## 5.2 Special hazards arising from the substance or mixture

No data available

## 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

## 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

For personal protection see section 8.

### 6.2 Environmental precautions

Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Moisture sensitive.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Trisodium orthophosphate	7601-54-9	STEL	5.000000 mg/m3	USA. Workplace Environmental Exposure Levels (WEEL)

### 8.2 Exposure controls

#### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### Personal protective equipment

##### Eye/face protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact  
Material: Nitrile rubber  
Minimum layer thickness: 0.11 mm  
Break through time: 480 min  
Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact  
Material: Nitrile rubber  
Minimum layer thickness: 0.11 mm  
Break through time: 480 min  
Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

### **Body Protection**

Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

### **Respiratory protection**

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

### **Control of environmental exposure**

Do not let product enter drains.

---

## **9. PHYSICAL AND CHEMICAL PROPERTIES**

### **9.1 Information on basic physical and chemical properties**

- |   |   |
|---|---|
| a) Appearance                                   | Form: solid                                 |
| b) Odour  | No data available                           |
| c) Odour Threshold                              | No data available                           |
| d) pH   | 11 at 20 °C (68 °F)                         |
| e) Melting point/freezing point                 | 75 °C (167 °F) - Decomposes before melting. |
| f) Initial boiling point and boiling range      | No data available                           |
| g) Flash point                                  | No data available                           |
| h) Evaporation rate                             | No data available                           |
| i) Flammability (solid, gas)                    | No data available                           |
| j) Upper/lower flammability or explosive limits | No data available                           |
| k) Vapour pressure                              | No data available                           |
| l) Vapour density                               | No data available                           |
| m) Relative density                             | No data available                           |
| n) Water solubility                             | 121 g/l at 20 °C (68 °F)                    |
| o) Partition coefficient: n-octanol/water       | No data available                           |
| p) Auto-ignition temperature                    | No data available                           |

- |                              |   |
|------------------------------|---|
| q) Decomposition temperature | 75 °C (167 °F) - Decomposes before melting. |
| r) Viscosity                 | No data available                           |
| s) Explosive properties      | No data available                           |
| t) Oxidizing properties      | No data available                           |

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong acids

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Oxides of phosphorus, Sodium oxides

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

No data available

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: Irritating to skin.

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Moderate eye irritation

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a

known or anticipated carcinogen by NTP.

No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

Inhalation - May cause respiratory irritation.

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: TC9490000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION**

**12.1 Toxicity**

No data available

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS**

**13.1 Waste treatment methods**

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION**

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION****SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

Acute Health Hazard

**Massachusetts Right To Know Components**

	CAS-No.	Revision Date
Trisodium orthophosphate	7601-54-9	2007-03-01

**Pennsylvania Right To Know Components**

	CAS-No.	Revision Date
Trisodium orthophosphate	7601-54-9	2007-03-01

**New Jersey Right To Know Components**

	CAS-No.	Revision Date
Trisodium orthophosphate	7601-54-9	2007-03-01

**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

**16. OTHER INFORMATION****Full text of H-Statements referred to under sections 2 and 3.**

Eye Irrit.	Eye irritation
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
Skin Irrit.	Skin irritation
STOT SE	Specific target organ toxicity - single exposure

**HMIS Rating**

Health hazard:	2
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

**NFPA Rating**

Health hazard:	2
Fire Hazard:	0
Reactivity Hazard:	0

**Further information**

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

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## SAFETY DATA SHEET

Version 6.13

Revision Date 10/03/2017

Print Date 04/08/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Sodium borohydride

Product Number : 452882

Brand : Aldrich

CAS-No. : 16940-66-2

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

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2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Substances and mixtures, which in contact with water, emit flammable gases (Category 1), H260

Acute toxicity, Oral (Category 3), H301

Skin corrosion (Category 1B), H314

Serious eye damage (Category 1), H318

Reproductive toxicity (Category 1B), H360

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Danger

Hazard statement(s)

H260

In contact with water releases flammable gases which may ignite spontaneously.

H301

Toxic if swallowed.

H314

Causes severe skin burns and eye damage.

H360

May damage fertility or the unborn child.

Precautionary statement(s)

P201

Obtain special instructions before use.

P202

Do not handle until all safety precautions have been read and understood.

P223

Do not allow contact with water.

P231 + P232	Handle under inert gas. Protect from moisture.
P260	Do not breathe dust or mist.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P310 + P330	IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.
P335 + P334	Brush off loose particles from skin. Immerse in cool water/ wrap in wet bandages.
P363	Wash contaminated clothing before reuse.
P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
P402 + P404	Store in a dry place. Store in a closed container.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

## 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.1 Substances

Synonyms	: Sodium tetrahydridoborate VenPure®
Formula	: H <sub>4</sub> BNa
Molecular weight	: 37.83 g/mol
CAS-No.	: 16940-66-2
EC-No.	: 241-004-4

#### Hazardous components

Component	Classification	Concentration
<b>Sodium borohydride</b>		
	Water-react. 1; Acute Tox. 3; Skin Corr. 1B; Eye Dam. 1; Repr. 1B; H260, H301, H314, H360F	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact**

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

**In case of eye contact**

Continue rinsing eyes during transport to hospital. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

**If swallowed**

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Dry powder

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust. For personal protection see section 8.

**6.2 Environmental precautions**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Sweep up and shovel. Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13). Do not flush with water. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs. Provide appropriate exhaust ventilation at places where dust is formed. Keep away from sources of ignition - No smoking. For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place. Never allow product to get in contact with water during storage.

Air and moisture sensitive. Store under inert gas. Keep in a dry place.

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

#### Personal protective equipment

##### Eye/face protection

Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

##### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

##### Body Protection

Complete suit protecting against chemicals, Flame retardant protective clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |                    |                               |
|--------------------|-------------------------------|
| a) Appearance      | Form: powder<br>Colour: white |
| b) Odour           | No data available             |
| c) Odour Threshold | No data available             |

d) pH	No data available
e) Melting point/freezing point	Melting point/range: > 300 °C (> 572 °F) - dec.
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	Lower explosion limit: 3.02 %(V)
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	No data available
n) Water solubility	55 g/l at 25 °C (77 °F) - soluble
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

## 9.2 Other safety information

Bulk density 1,074 kg/m<sup>3</sup> at 20 °C (68 °F)

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

Reacts violently with water.

### 10.4 Conditions to avoid

Exposure to moisture

### 10.5 Incompatible materials

Oxidizing agents, Chemically active metals, acids, Reacts violently with water.

### 10.6 Hazardous decomposition products

Other decomposition products - No data available

Hazardous decomposition products formed under fire conditions. - Borane/boron oxides, Sodium oxides

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD<sub>50</sub> Oral - Rat - 162 mg/kg

Inhalation: No data available

LD50 Dermal - Rabbit - 4,000 - 8,000 mg/kg

No data available

**Skin corrosion/irritation**

Skin - Human

Result: Corrosive

**Serious eye damage/eye irritation**

Eyes - Human

Result: Corrosive to eyes

**Respiratory or skin sensitisation**

No data available

**Germ cell mutagenicity**

No data available

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

Clear evidence of adverse effects on sexual function and fertility, based on animal experiments.

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: ED3325000

Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin., spasm, inflammation and edema of the larynx, spasm, inflammation and edema of the bronchi, pneumonitis, pulmonary edema, burning sensation, Cough, wheezing, laryngitis, Shortness of breath, Headache, Nausea, Vomiting, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

## 12. ECOLOGICAL INFORMATION

### 12.1 Toxicity

Toxicity to fish                      mortality LC50 - Gambusia affinis (Mosquito fish) - 5,600 mg/l - 96 h

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

### 12.6 Other adverse effects

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

#### Product

Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

#### Contaminated packaging

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

#### DOT (US)

UN number: 1426      Class: 4.3      Packing group: I  
Proper shipping name: Sodium borohydride  
Reportable Quantity (RQ):  
Poison Inhalation Hazard: No

#### IMDG

UN number: 1426      Class: 4.3      Packing group: I      EMS-No: F-G, S-O  
Proper shipping name: SODIUM BOROHYDRIDE

#### IATA

UN number: 1426      Class: 4.3      Packing group: I  
Proper shipping name: Sodium borohydride  
IATA Passenger: Not permitted for transport

---

## 15. REGULATORY INFORMATION

#### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

#### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

#### SARA 311/312 Hazards

Reactivity Hazard, Acute Health Hazard, Chronic Health Hazard

#### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

#### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Sodium borohydride	16940-66-2	2007-03-01

#### New Jersey Right To Know Components

	CAS-No.	Revision Date
Sodium borohydride	16940-66-2	2007-03-01

#### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

#### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Dam.	Serious eye damage
H260	In contact with water releases flammable gases which may ignite spontaneously.



H301	Toxic if swallowed.
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H360	May damage fertility or the unborn child.
H360F	May damage fertility.
Repr.	Reproductive toxicity
Skin Corr.	Skin corrosion

#### HMIS Rating

Health hazard:	3
Chronic Health Hazard:	*
Flammability:	4
Physical Hazard	2

#### NFPA Rating

Health hazard:	3
Fire Hazard:	4
Reactivity Hazard:	2
Special hazard.I:	W

#### Further information

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

#### Preparation Information

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 6.13

Revision Date: 10/03/2017

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 5.10  
Revision Date 10/02/2017  
Print Date 03/24/2018

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : Sodium chloride

Product Number : S7653  
Brand : Sigma-Aldrich

CAS-No. : 7647-14-5

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Synthesis of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture.

**2.2 GHS Label elements, including precautionary statements**

Not a hazardous substance or mixture.

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none**

---

**3. COMPOSITION/INFORMATION ON INGREDIENTS****3.1 Substances**

Formula : NaCl  
Molecular weight : 58.44 g/mol  
CAS-No. : 7647-14-5  
EC-No. : 231-598-3

No components need to be disclosed according to the applicable regulations.

---

**4. FIRST AID MEASURES****4.1 Description of first aid measures****General advice**

Consult a physician. Show this safety data sheet to the doctor in attendance.

**If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact**

Wash off with soap and plenty of water. Consult a physician.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Avoid breathing dust. For personal protection see section 8.

**6.2 Environmental precautions**

Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Avoid formation of dust and aerosols. Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

#### Personal protective equipment

##### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

##### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

##### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |                           |  |
|---------------------------|--|
| a) Appearance             | Form: solid<br>Colour: colourless      |
| b) Odour                  | No data available                      |
| c) Odour Threshold        | No data available                      |
| d) pH                     | 7                                      |
| e) Melting point/freezing | Melting point/range: 801 °C (1,474 °F) |

point

- |   |   |
|---|---|
| f) Initial boiling point and boiling range      | 1,413 °C (2,575 °F)                       |
| g) Flash point                                  | No data available                         |
| h) Evaporation rate                             | No data available                         |
| i) Flammability (solid, gas)                    | No data available                         |
| j) Upper/lower flammability or explosive limits | No data available                         |
| k) Vapour pressure                              | 1.33 hPa (1.00 mmHg) at 865 °C (1,589 °F) |
| l) Vapour density                               | No data available                         |
| m) Relative density                             | 2.1650 g/cm <sup>3</sup>                  |
| n) Water solubility                             | 358 g/l at 20 °C (68 °F) - soluble        |
| o) Partition coefficient: n-octanol/water       | No data available                         |
| p) Auto-ignition temperature                    | No data available                         |
| q) Decomposition temperature                    | No data available                         |
| r) Viscosity                                    | No data available                         |
| s) Explosive properties                         | No data available                         |
| t) Oxidizing properties                         | No data available                         |

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Hydrogen chloride gas, Sodium oxides

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD<sub>50</sub> Oral - Rat - 3,550 mg/kg

LC<sub>50</sub> Inhalation - Rat - 1 h - > 42,000 mg/m<sup>3</sup>

LD<sub>50</sub> Dermal - Rabbit - > 10,000 mg/kg

No data available

**Skin corrosion/irritation**

No data available

**Serious eye damage/eye irritation**

No data available

**Respiratory or skin sensitisation**

No data available

**Germ cell mutagenicity**

No data available

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: VZ4725000

Vomiting, Diarrhoea, Dehydration and congestion may occur in internal organs. Hypertonic salt solutions can produce inflammatory reactions in the gastrointestinal tract., To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

Toxicity to fish LC50 - *Lepomis macrochirus* (Bluegill) - 5,840 mg/l - 96 h

Toxicity to daphnia and other aquatic invertebrates NOEC - *Daphnia* (water flea) - 1,500 mg/l - 7 d

LC50 - *Daphnia magna* (Water flea) - 1,661 mg/l - 48 h

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

## 13. DISPOSAL CONSIDERATIONS

### 13.1 Waste treatment methods

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

## 15. REGULATORY INFORMATION

**SARA 302 Components**

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

No SARA Hazards

**Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

**Pennsylvania Right To Know Components**

Sodium chloride	CAS-No. 7647-14-5	Revision Date
-----------------	----------------------	---------------

**New Jersey Right To Know Components**

Sodium chloride	CAS-No. 7647-14-5	Revision Date
-----------------	----------------------	---------------

**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

**HMIS Rating**

Health hazard:	1
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

**NFPA Rating**

Health hazard:	1
Fire Hazard:	0
Reactivity Hazard:	0

**Further information**

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the

product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 5.10

Revision Date: 10/02/2017

Print Date: 03/24/2018



## SAFETY DATA SHEET

Version 3.8

Revision Date 10/20/2017

Print Date 04/08/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Sodium citrate dihydrate

Product Number : W302600

Brand : Aldrich

CAS-No. : 6132-04-3

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

## 2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Synonyms : Sodium citrate tribasicdihydrate  
Trisodium citratedihydrate  
Citric acidtrisodium salt dihydrateFormula :  $C_6H_5Na_3O_7 \cdot 2H_2O$ 

Molecular weight : 294.1 g/mol

CAS-No. : 6132-04-3

EC-No. : 200-675-3

Registration number : 01-2119457027-40-XXXX

No components need to be disclosed according to the applicable regulations.

---

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### **If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

#### **In case of skin contact**

Wash off with soap and plenty of water.

#### **In case of eye contact**

Flush eyes with water as a precaution.

#### **If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

---

## 5. FIREFIGHTING MEASURES

### 5.1 Extinguishing media

#### **Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

### 5.2 Special hazards arising from the substance or mixture

No data available

### 5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information

No data available

---

## 6. ACCIDENTAL RELEASE MEASURES

### 6.1 Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

### 6.2 Environmental precautions

Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

### 6.4 Reference to other sections

For disposal see section 13.

---

## 7. HANDLING AND STORAGE

### 7.1 Precautions for safe handling

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

Keep in a dry place.

Storage class (TRGS 510): 13: Non Combustible Solids

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

General industrial hygiene practice.

#### Personal protective equipment

##### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

##### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

##### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

##### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |                                 |   |
|---------------------------------|---|
| a) Appearance                   | Form: powder<br>Colour: white                   |
| b) Odour                        | No data available                               |
| c) Odour Threshold              | No data available                               |
| d) pH                           | 7.5 - 9 at 29.4 g/l at 25 °C (77 °F)            |
| e) Melting point/freezing point | Melting point/range: > 300 °C (> 572 °F) - lit. |

f) Initial boiling point and boiling range	309.6 °C (589.3 °F) at 1,013 hPa (760 mmHg)
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	No data available
n) Water solubility	29.4 g/l at 20 °C (68 °F) - completely soluble
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Sodium oxides

Other decomposition products - No data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

No data available

Inhalation: No data available

Dermal: No data available

No data available

**Skin corrosion/irritation**

No data available

**Serious eye damage/eye irritation**

No data available

**Respiratory or skin sensitisation**

No data available

**Germ cell mutagenicity**

No data available

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

No data available

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**  
Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

### DOT (US)

Not dangerous goods

### IMDG

Not dangerous goods

### IATA

Not dangerous goods

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

No SARA Hazards

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

CITRIC ACID TRISODIUM SALT Dihydrate	CAS-No. 6132-04-3	Revision Date
--------------------------------------	----------------------	---------------

### New Jersey Right To Know Components

CITRIC ACID TRISODIUM SALT Dihydrate	CAS-No. 6132-04-3	Revision Date
--------------------------------------	----------------------	---------------

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

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## 16. OTHER INFORMATION

### HMIS Rating

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 3.8

Revision Date: 10/20/2017

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 4.6  
Revision Date 09/11/2015  
Print Date 04/08/2018

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1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : *N,N,N',N'*-Tetramethylethylenediamine

Product Number : T7024  
Brand : Sigma  
Index-No. : 612-103-00-3

CAS-No. : 110-18-9

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

**GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)**

Flammable liquids (Category 2), H225  
Acute toxicity, Oral (Category 3), H301  
Acute toxicity, Inhalation (Category 3), H331  
Skin corrosion (Category 1B), H314  
Serious eye damage (Category 1), H318

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 2.2 GHS Label elements, including precautionary statements

Pictogram



Signal word

Danger

Hazard statement(s)

H225

Highly flammable liquid and vapour.

H301 + H331

Toxic if swallowed or if inhaled

H314

Causes severe skin burns and eye damage.

Precautionary statement(s)

P210

Keep away from heat/sparks/open flames/hot surfaces. No smoking.

P233

Keep container tightly closed.

P240

Ground/bond container and receiving equipment.

P241

Use explosion-proof electrical/ ventilating/ lighting/ equipment.



P242	Use only non-sparking tools.
P243	Take precautionary measures against static discharge.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.
P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/ physician.
P321	Specific treatment (see supplemental first aid instructions on this label).
P363	Wash contaminated clothing before reuse.
P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P403 + P235	Store in a well-ventilated place. Keep cool.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

## 3. COMPOSITION/INFORMATION ON INGREDIENTS

### 3.1 Substances

Synonyms : TMEDA  
1,2-Bis(dimethylamino)ethane  
TEMED

Formula : C<sub>6</sub>H<sub>16</sub>N<sub>2</sub>  
Molecular weight : 116.2 g/mol  
CAS-No. : 110-18-9  
EC-No. : 203-744-6  
Index-No. : 612-103-00-3

#### Hazardous components

Component	Classification	Concentration
<b>N,N,N',N'-Tetramethylethylenediamine</b>		
	Flam. Liq. 2; Acute Tox. 3; Skin Corr. 1B; Eye Dam. 1; H225, H301 + H331, H314	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

## 4. FIRST AID MEASURES

### 4.1 Description of first aid measures

#### General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

#### If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

#### In case of skin contact

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

**In case of eye contact**

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital.

**If swallowed**

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

Use water spray to cool unopened containers.

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas. For personal protection see section 8.

**6.2 Environmental precautions**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Use explosion-proof equipment. Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge. For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Handle and store under inert gas. Air and moisture sensitive.

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

### 8.1 Control parameters

#### Components with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

#### Appropriate engineering controls

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

#### Personal protective equipment

##### Eye/face protection

Tightly fitting safety goggles. Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

##### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.4 mm

Break through time: 125 min

Material tested: Camatril® (KCL 730 / Aldrich Z677442, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

##### Body Protection

Complete suit protecting against chemicals, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

##### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

##### Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |  |   |
|--|---|
| a) Appearance                              | Form: liquid, clear<br>Colour: colourless |
| b) Odour                                   | No data available                         |
| c) Odour Threshold                         | No data available                         |
| d) pH                                      | No data available                         |
| e) Melting point/freezing point            | Melting point/range: -55 °C (-67 °F)      |
| f) Initial boiling point and boiling range | 120 - 122 °C (248 - 252 °F)               |
| g) Flash point                             | 20 °C (68 °F) - closed cup                |

h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	Upper explosion limit: 9.08 %(V) Lower explosion limit: 0.98 %(V)
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	0.775 g/mL at 20 °C (68 °F)
n)	Water solubility	soluble
o)	Partition coefficient: n-octanol/water	log Pow: 0.3
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

## 9.2 Other safety information

No data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

Vapours may form explosive mixture with air.

### 10.4 Conditions to avoid

Heat, flames and sparks. Extremes of temperature and direct sunlight.

### 10.5 Incompatible materials

Strong oxidizing agents, Carbon dioxide (CO<sub>2</sub>), Copper

### 10.6 Hazardous decomposition products

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD50 Oral - Rat - 268 mg/kg

LC50 Inhalation - Rat - 4 h - 1318 ppm

LD50 Dermal - Rabbit - 5,390 mg/kg

No data available

#### Skin corrosion/irritation

No data available

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: Severe eye irritation

**Respiratory or skin sensitisation**

No data available

**Germ cell mutagenicity**

No data available

**Carcinogenicity**

- IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
- ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
- NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
- OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: KV7175000

Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin., spasm, inflammation and edema of the larynx, spasm, inflammation and edema of the bronchi, pneumonitis, pulmonary edema

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

No data available

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

**Contaminated packaging**  
Dispose of as unused product.

---

## 14. TRANSPORT INFORMATION

### DOT (US)

UN number: 3286      Class: 3 (6.1, 8)      Packing group: II  
Proper shipping name: Flammable liquid, toxic, corrosive, n.o.s.

Poison Inhalation Hazard: No

### IMDG

UN number: 2372      Class: 3      Packing group: II      EMS-No: F-E, S-D  
Proper shipping name: 1,2-DI-(DIMETHYLAMINO)ETHANE

### IATA

UN number: 2372      Class: 3      Packing group: II  
Proper shipping name: 1,2-Di-(dimethylamino) ethane

---

## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

Fire Hazard, Acute Health Hazard

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

N,N,N',N'-Tetramethylethylenediamine	CAS-No. 110-18-9	Revision Date 2007-03-01
--------------------------------------	---------------------	-----------------------------

### New Jersey Right To Know Components

N,N,N',N'-Tetramethylethylenediamine	CAS-No. 110-18-9	Revision Date 2007-03-01
--------------------------------------	---------------------	-----------------------------

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

## 16. OTHER INFORMATION

### Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Dam.	Serious eye damage
Flam. Liq.	Flammable liquids
H225	Highly flammable liquid and vapour.
H301	Toxic if swallowed.
H301 + H331	Toxic if swallowed or if inhaled
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.

### HMIS Rating

Health hazard:	3
Chronic Health Hazard:	
Flammability:	3

Physical Hazard 0

**NFPA Rating**

Health hazard: 3

Fire Hazard: 3

Reactivity Hazard: 0

**Further information**

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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

**Preparation Information**

Sigma-Aldrich Corporation

Product Safety – Americas Region

1-800-521-8956

Version: 4.6

Revision Date: 09/11/2015

Print Date: 04/08/2018

## SAFETY DATA SHEET

Version 5.0

Revision Date 01/16/2018

Print Date 04/10/2018

---

1. PRODUCT AND COMPANY IDENTIFICATION

## 1.1 Product identifiers

Product name : Urea (200 mg)

Product Number : 1706698

Brand : USP

CAS-No. : 57-13-6

## 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

## 1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832

Fax : +1 800-325-5052

## 1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

---

2. HAZARDS IDENTIFICATION

## 2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

## 2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

## 2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

## 3.1 Substances

Molecular weight : 60.06 g/mol

CAS-No. : 57-13-6

## Hazardous components

Component	Classification	Concentration
Urea		
		90 - 100 %

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4. FIRST AID MEASURES

## 4.1 Description of first aid measures

## If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration.



**In case of skin contact**

Wash off with soap and plenty of water.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

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**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

No data available

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

No data available

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**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

**6.2 Environmental precautions**

No special environmental precautions required.

**6.3 Methods and materials for containment and cleaning up**

Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

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**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

Storage class (TRGS 510): 13: Non Combustible Solids

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

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**8. EXPOSURE CONTROLS/PERSONAL PROTECTION****8.1 Control parameters**

Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Urea	57-13-6	TWA	10.000000 mg/m3	USA. Workplace Environmental Exposure Levels (WEEL)

## 8.2 Exposure controls

### Appropriate engineering controls

General industrial hygiene practice.

### Personal protective equipment

#### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

#### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

#### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

#### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Control of environmental exposure

No special environmental precautions required.

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- |   |   |
|---|---|
| a) Appearance                                   | Form: solid<br>Colour: white                  |
| b) Odour  | No data available                             |
| c) Odour Threshold                              | No data available                             |
| d) pH   | 7.5 - 9.5 at 480 g/l at 25 °C (77 °F)         |
| e) Melting point/freezing point                 | 133 - 135 °C (271 - 275 °F)                   |
| f) Initial boiling point and boiling range      | No data available                             |
| g) Flash point                                  | No data available                             |
| h) Evaporation rate                             | No data available                             |
| i) Flammability (solid, gas)                    | No data available                             |
| j) Upper/lower flammability or explosive limits | No data available                             |
| k) Vapour pressure                              | 0.009 hPa (0.007 mmHg) at 20 °C (68 °F)       |
| l) Vapour density                               | No data available                             |
| m) Relative density                             | 1.335 g/cm3                                   |
| n) Water solubility                             | 480 g/l at 20 °C (68 °F) - completely soluble |
| o) Partition coefficient: n-octanol/water       | log Pow: -2.59 - -1.59                        |

p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

## 9.2 Other safety information

Bulk density 700 - 800 kg/m<sup>3</sup> at 20 °C (68 °F)

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## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

No data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

No data available

### 10.4 Conditions to avoid

No data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NO<sub>x</sub>)  
In the event of fire: see section 5

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## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

LD<sub>50</sub> Oral - Rat - 8,471 mg/kg

Inhalation: No data available

Dermal: No data available

No data available

#### Skin corrosion/irritation

Skin - Rabbit

Result: No skin irritation

#### Serious eye damage/eye irritation

Eyes - Rabbit

Result: No eye irritation

#### Respiratory or skin sensitisation

No data available

#### Germ cell mutagenicity

No data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is on OSHA's

list of regulated carcinogens.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

No data available

**Aspiration hazard**

No data available

**Additional Information**

RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

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**12. ECOLOGICAL INFORMATION**

**12.1 Toxicity**

Toxicity to fish LC50 - *Poecilia reticulata* (guppy) - 17,500 mg/l - 96 h

Toxicity to daphnia and other aquatic invertebrates EC50 - *Daphnia magna* (Water flea) - 3,910 mg/l - 48 h

**12.2 Persistence and degradability**

No data available

**12.3 Bioaccumulative potential**

No data available

**12.4 Mobility in soil**

No data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

No data available

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**13. DISPOSAL CONSIDERATIONS**

**13.1 Waste treatment methods**

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

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**14. TRANSPORT INFORMATION**

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

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## 15. REGULATORY INFORMATION

### SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

### SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

### SARA 311/312 Hazards

No SARA Hazards

### Massachusetts Right To Know Components

No components are subject to the Massachusetts Right to Know Act.

### Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Urea	57-13-6	

	CAS-No.	Revision Date
Urea	57-13-6	

### New Jersey Right To Know Components

	CAS-No.	Revision Date
Urea	57-13-6	

### California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

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## 16. OTHER INFORMATION

### HMIS Rating

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

### NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

### Further information

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### Preparation Information

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